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THE STAIRCASE PHENOMENON IN VENTRICULAR MUSCLE.

By ALISON S. DALE (*Yarrow Student, Girton College*).

(*From the Physiological Laboratory, Cambridge.*)

In a previous paper [Dale, 1930] a relation between the rhythm and the amplitude of contraction of mammalian (rabbit) ventricular muscle was described. It was found that when the natural rhythm of the perfused rabbit's heart is slowed, the amplitude of the contractions decreases. The same relation was found in the case of perfused ventricular strips. Such strips do not contract spontaneously, and it was shown that, when an artificial rhythm applied to them was halved, the amplitude of the contractions decreased, and returned to its former value when the rhythm was restored to its original rate. It is evident, therefore, that the phenomenon is a property of the ventricular muscle itself, and in the paper referred to above it was ascribed to a form of the staircase phenomenon, first described by Bowditch [1871].

It seemed desirable to make a further study of the phenomenon, and to determine, if possible, the conditions necessary for its appearance.

Conditions of perfusion.

Experience showed that the phenomenon was always present in a strip perfused with a buffered Ringer's solution, provided that the muscle was in good condition. In a feebly beating strip it was not always evident and might even be reversed. Feeble contractions were usually caused by a slow rate of perfusion, and consequent insufficient supply of oxygen. Slowing of the rhythm must in any case increase the amount of oxygen available for each contraction. In cases, therefore, in which the supply is inadequate at a fast rhythm, slowing of the rhythm would be expected to have a beneficial effect on the contractions, masking the decrease observed in normal preparations.

As the phenomenon had only been observed in preparations perfused with a modified Ringer's solution, it seemed possible that it might be

peculiar to the perfused muscle. Some experiments were therefore carried out in which the perfusion fluid was a mixture of blood and Ringer's solution. The Ringer's solution used was buffered with bicarbonate, and was made isotonic for the blood corpuscles by reducing the sodium chloride content. The blood (about 40 c.c.) was obtained from a rabbit by decapitation, whipped, filtered through muslin, and made up to 1000 c.c. with the bicarbonate Ringer's solution. The mixture was then filtered through cotton-wool to remove any shreds of clot which might have passed the muslin. The heart of the same rabbit was used for making the ventricular strip preparation. The perfusion and stimulation apparatus were the same as those described in the previous paper. Perfusion was started with Ringer's solution alone, which was replaced by the mixture of blood and Ringer's solution as soon as this was prepared. Immediately the blood reached the strip, there was an increase in the amplitude of the contractions. This beneficial effect of the blood did not persist however. It was found that after the first increase of amplitude, the contractions became progressively weaker, until they scarcely moved the lever. The cause of the weakening was a gradual decrease in the rate of perfusion, terminating in a nearly complete stoppage of the flow. The reason for this stoppage is not clear, but it seems to be peculiar to the strip preparation, as it is not observed in whole hearts perfused with mixtures of blood and Ringer, or whole blood, though Cushny and Gunn [1913] observed it in whole hearts perfused with mixtures of serum and Ringer. It is possible that, in the strips, the blood corpuscles may accumulate at the cut ends of the coronary capillaries, and block the outflow.

In any case, the onset of this weakening was slow enough to permit observations being made while the excursions were still of reasonable size. Halving of the rhythm then produced the usual decrease in amplitude of the contractions, and subsequent restoration of the original rhythm the return to normal.

Effect of changing pH of perfusate.

A series of experiments was next carried out with a view to determining whether the relation between rhythm and amplitude could be changed by variations in the composition of the perfusion fluid. Adrian's experiments [1920] on the perfused ventricle of the frog showed that, while at acid reactions a contraction is followed by a short period during which a second contraction is supernormal, this supernormal phase disappears when the perfusion fluid is made alkaline. Adrian concluded

that the presence of the supernormal phase is necessary for the production of the Bowditch staircase. Although, as was pointed out in the previous paper, there is no supernormal phase following a contraction of the rabbit's ventricle, it seemed possible that the supposed staircase effect might be influenced by changes in the reaction of the perfusion fluid.

The strip preparation was again employed. The Ringer's solution was buffered with borate and acetate (H_3BO_3 0·062 p.c., CH_3COONa 0·082 p.c., NaCl 0·8 p.c., KCl 0·04 p.c., $CaCl_2$ 0·021 p.c.) and was brought to the required *p*H by addition of the appropriate quantity of HCl or NaOH, comparison being made with a standard buffer solution, using phenol red as indicator. Perfusion was always started with a solution at *p*H 7·5, and this was followed by fluids of more acid and more alkaline reactions. When changing to a solution of a different reaction, half an hour of perfusion with the new fluid was allowed before any records were taken. A record consisted of a series of contractions at the full rhythm, followed by a series at the halved rhythm, and finally a return to the full rhythm. The full rhythm was usually 60 beats per min. This is slow when compared with the natural rhythm of the rabbit's heart, but at the relatively slow rate of perfusion in the strips it is the maximum possible without production of fatigue. Two or three records were taken at each reaction. The excursions of the lever at the full and halved rhythms were then measured, and the decrease in amplitude expressed as the ratio of the excursion at the halved rhythm to that at the full rhythm. It sometimes happened that, when the rhythm was halved, the decrease in amplitude produced was not maintained, but gave place to a gradual increase. This was, no doubt, the result of inefficient perfusion (see above), the beneficial effect of slower rhythm, under these conditions, appearing after a short delay. In such cases the value for the amplitude at the halved rhythm was taken as the minimum reached before the secondary increase set in.

Table I shows a few results typical of those obtained.

It is evident from Table I that there is no significant change in the ratio H/F when a neutral perfusion fluid is replaced by an alkaline one. The small changes which occur are not always in the same direction, and in any case are no greater than the variations among the several values obtained for the same reaction. Figures from records obtained at an acid reaction are not given, as they, also, show no significant variation from normal.

These results are not in direct disagreement with those obtained by Adrian for two reasons. Firstly, the range of reactions employed

TABLE I. Results from two experiments. F is the height in mm. of the record of the contractions at the full rate, H that at the halved rate.
Values for consecutive observations in each experiment.

No. of exp.	Temp. °C.	pH	F	H	H/F
1	33	7·4	10·5	9·2	0·86
		7·4	11·6	10·2	0·88
		7·4	12·5	10·9	0·87
		8·1	9·9	9·0	0·90
		8·1	9·9	8·9	0·89
		7·6	8·2	7·2	0·88
		7·6	9·0	7·6	0·84
2	33	7·6	15·0	13·0	0·87
		7·6	13·0	11·7	0·84
		8·2	11·7	10·0	0·85
		8·2	11·8	10·2	0·86
		8·2	12·0	10·3	0·86
		8·2	12·2	10·2	0·84
		7·6	12·7	10·9	0·86
		7·6	12·7	11·1	0·87

by him was much greater. The rabbit's heart is much more sensitive to changes in composition of the perfusion fluid than that of the frog, and larger changes in reaction would have rendered the muscle so hypotonic, and the excursions of the lever so small, that accurate measurement of the curves would have been impossible. Secondly, as pointed out above, Adrian's experiments were concerned with the supernormal phase, which is not present in rabbit's heart muscle.

Changes in Ca˙ and K˙ ion content.

It seemed possible that, as the Ca˙ and K˙ ions have such a profound influence on the contraction of cardiac muscle, alteration of the concentration of these ions in the perfusion fluid might produce some change in the relation between rhythm and amplitude of contraction. Experiments were therefore carried out in which the concentrations of the ions were varied. Solutions were made up, containing normal, halved or doubled contractions of Ca˙ or K˙ ion, and records were taken in the same way as those described above. The records were measured, and the ratio H/F calculated, but it was found that no change in the ratio was produced by the variations in concentration of the ions.

The staircase phenomenon in the frog's heart.

Most of the previous work on the staircase phenomenon in cardiac muscle has been carried out on the heart of the frog. Many of the observations have been made on the heart *in situ*, others on the perfused

organ. It seems that the staircase phenomenon is not always apparent, and it has become customary to refer to "Treppenbedingungen," *i.e.* the conditions necessary for the appearance of the staircase effect. In the experiments on the rabbit's ventricle just described, these staircase conditions seem to be the normal conditions of experiment, and abnormal ones as well, since it was found impossible to abolish the staircase by any change in the perfusion fluid which was tried. Conditions of fatigue, produced by slow perfusion, were the only ones in which the staircase disappeared.

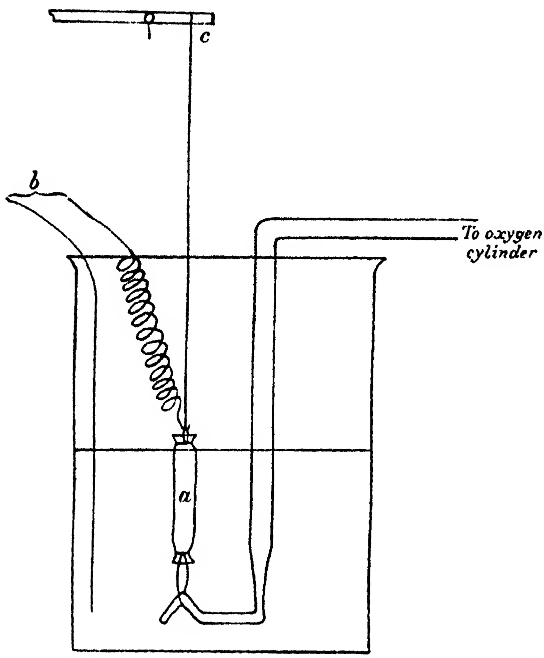

Fig. 1. Isolated strip preparation of frog's ventricle. *a* is the strip; *b*, stimulating electrodes; *c*, lever.

It seemed desirable, therefore, to make some observations on the cardiac muscle of the frog, to determine, if possible, what the nature of these staircase conditions might be. It was necessary that the preparation used should have no rhythm of its own, in order that the rate of beating might be artificially regulated. A strip preparation was therefore employed, and was obtained as follows. The frog was pithed, and the heart removed and placed in Ringer's solution, where it was allowed to beat for a few minutes to wash the blood from it. The auricles were then

cut away at the A.V. groove, and a cut was made across the ventricle, parallel to the groove, and about 2 mm. below it. A ring of ventricular muscle was thus obtained, and this, when cut open, formed the strip. A piece of silk was tied to each end of the strip, one end being secured to a fixed point, and the other end to the lever. The strip was immersed in a beaker of buffered Ringer's solution, through which oxygen was

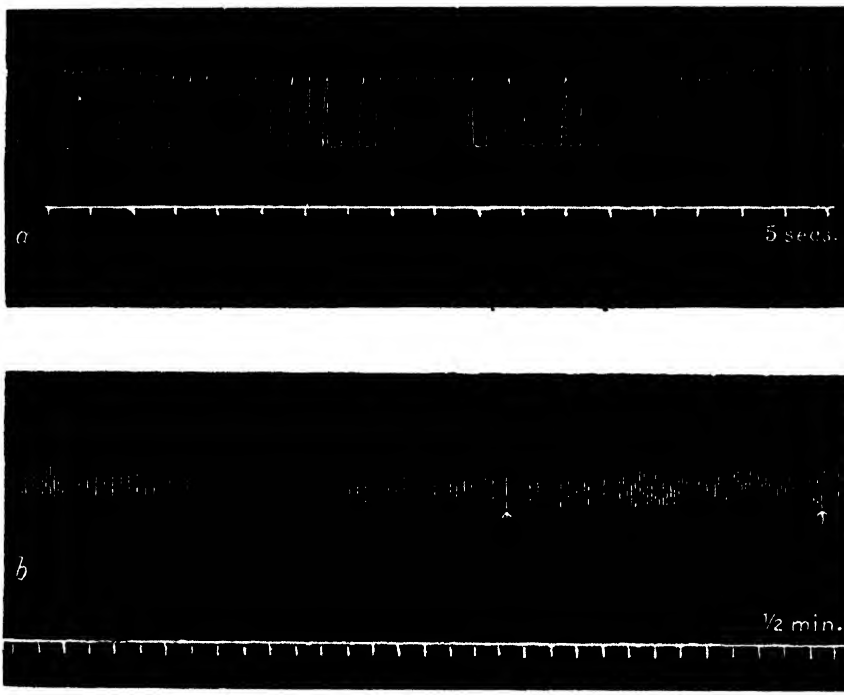

Fig. 2. *a*, record from rabbit's ventricular strip. Effect of halve-rhythm and then restoring it to normal. *b*, record from frog's ventricular strip. Rhythm halved and then restored to normal. ↑ indicate stopping of drum for 2 min.

bubbled. This served both to oxygenate and stir the solution. The solution was made up according to the formula given by Anrep and Segall [1926], this having the advantage that, by mixture of appropriate proportions of two solutions, a fluid of any desired reaction can be obtained. In the first series of experiments the reaction was adjusted to *p*H 7·5. Fig. 1 shows the arrangement of the apparatus.

The strip was stimulated rhythmically by means of the apparatus employed in the experiments on the rabbit's ventricle, but the motor

was geared down so that the full rhythm was 12 beats per min. This rhythm is very near the optimum for the frog's ventricle at room temperature, fatigue setting in at rates above 16 per min. The presence of the staircase was tested by observing the effect of halving the rhythm. Fig. 2*b* is a typical record, Fig. 2*a* being a record from a rabbit's ventricular strip inserted for comparison.

It is evident that the halving of the rhythm produces a decrease in the amplitude of contraction in the frog's ventricular muscle, as it does in the rabbit's, but the process differs in two respects. In the first place, the duration of the actual "staircase," whether descending or ascending, is much longer in the frog's heart than it is in the rabbit's, in respect of both time and number of beats. This is easily accounted for on the ground that all processes in the frog are slower than those in the mammal. The second difference is more important. It was a striking fact, obvious in the whole heart, and in the ventricular strip of the rabbit, that when the rhythm became slower, the first beat at the lower rate was enlarged, and was then followed by the descending staircase of beats. Again, when the rate changed from a low to a high one, the first beat at the high rate was always smaller than its predecessor, and was followed by an ascending staircase of beats. In the frog's heart these anomalous beats do not occur. On halving the rhythm, the decrease of amplitude begins immediately, and on doubling it, the increase begins with the first beat at the new rhythm. The frog's cardiac muscle therefore differs from that of the rabbit in that, as shown by Adrian, a true supernormal phase follows each contraction.

Attempts to abolish the staircase.

Various authors have described abolition or reversal of the staircase phenomenon. Adrian, as mentioned above, found that the supernormal phase disappeared when the heart was perfused with an alkaline solution, while Bowditch [1871] and Bornstein [1906] both claim to have produced a reversal of the staircase with atropine. In view of the fact that it had been found impossible to abolish the staircase in strips of rabbit's ventricle, except by defect of oxygen, it was decided to repeat experiments with alkali and atropine on the frog ventricular strips. In neither case did the staircase disappear. When the rhythm was halved the amplitude of the beats still decreased, and increased again on return to the full rhythm, and there was also always an obvious staircase when contractions were resumed after a pause of 30 sec. or more.

In the case of atropine, a possible explanation of the discrepancy

between my results and those of Bowditch and Bornstein, lies in the fact that the concentration of atropine used by them was relatively enormous. Bowditch, using an isolated frog's ventricle filled with fluid, placed 0·6 mg. of atropine sulphate in the ventricular cavity, which, assuming the capacity of the ventricle to be 0·1 c.c., would produce a contraction of 0·6 p.c. Bornstein applied the drug to the heart *in situ* by dropping a 1 p.c. solution of atropine sulphate on its surface. In the present experiments, the addition of a few drops of a 1 p.c. solution to the fluid bathing the muscle, was found to be sufficient to produce a very definite increase in the amplitude of the contractions. The volume of the fluid in the bath was 50 c.c., so that the addition of two drops (0·1 c.c.) of a 1 p.c. solution produced a resultant concentration of atropine of 0·002 p.c. If larger quantities (0·5–1·0 c.c.) of the 1 p.c. solution of the drug were added, the muscle was rendered inexcitable, and the experiment could not be continued. Fiddes [1929] has found that atropine does not always abolish the staircase in the perfused frog's heart.

The discrepancy between the present results and those obtained by Adrian with alkaline perfusion fluid is more difficult to explain. Adrian's observations were made on the first two beats of the staircase only, and it seemed possible that, in the present series, the supernormal phase might disappear at the alkaline reaction, but the subsequent beats might show an increase, on account of a cumulative action of the contractions, such as that suggested to explain the staircase in the rabbit's ventricle. Inspection of the records, however, showed that this was not the case, the supernormal phase being quite evident even at *p*H 10.

There was the further possibility that the difference might lie in the preparation, as Adrian had used whole frog's hearts to which a Stannius ligature had been applied, after perfusion with Ringer's solution of the desired reaction. A few experiments were therefore performed, in which the whole ventricle was perfused, and its contractions recorded "isochorically" in the manner described by Anrep and Segall [1926]. The ventricle was stimulated rhythmically at a rate of 12 per min. and the staircase was studied after pauses in the stimulation of 90 sec. It was found, in confirmation of Adrian's results, that perfusion with an alkaline fluid (*p*H 9) abolished the staircase, the beats now being of equal size, or showing, at the most, a very slight gradual increase. In a further series of experiments a cannula was tied into the ventricle by a thread passing round the A.V. groove, thus rendering the ventricle quiescent, but passing underneath the aorta, so that this was left free for escape

of the perfusion fluid. The contractions were recorded by a lever attached by a thread to the ventricular apex. Three successful experiments of this type were performed, but in only one of them did perfusion with alkaline fluid produce any change. In the other two, supernormal phase and staircase remained, thus eliminating the possibility that the persistence of staircase in strips at alkaline reactions is due to cutting the ventricular muscle. Fiddes [1929] also found that perfusion with alkaline Ringer's solution does not always abolish the staircase. He further showed that, when it does, the staircase can be made to reappear by poisoning with various drugs. It seems therefore, that the $H^{\cdot}$ ion concentration cannot be the sole determining factor in production of the supernormal phase and staircase phenomenon. Gasser and Erlanger [1930] have shown that immersion in alkaline Ringer's solution does not always abolish that phase of the action current in nerve, which is associated with the supernormal phase of excitability.

Fiddes [1929] found that staircase is abolished by halving the NaCl content of the perfusion fluid. This was tried on the strip preparation in one experiment, and it was found that though no staircase was evident when the stimulation had been interrupted for 1 min., after pauses of 4, 5 and 10 min. there was an obvious staircase. The reduction of the first beat was, however, much less than it would have been, had the perfusion fluid contained the normal concentration of NaCl. Halving the NaCl content does not abolish the staircase therefore, but decreases its intensity.

The cause of the staircase.

Several writers have brought forward theories to explain the appearance of the staircase phenomenon. Lee [1907] showed that asphyxia, or perfusion with fluids of acid reaction, caused an increase in the contractions of rhythmically stimulated skeletal muscles, and that the increase took the form of an ascending staircase of contractions. He concluded that the staircase was produced by an accumulation of metabolites (CO_2, lactic acid) which favoured an increase in the amplitude of contraction. Mines [1913] put forward a similar theory to explain the occurrence of an optimal rhythm and staircase in cardiac muscle. He concluded that the pH of resting muscle is on the alkaline side of the optimal, but that at a definite interval after a contraction, the optimal interval, the diffusion through the tissue of the acid produced by the contraction will bring the reaction of the tissue to the optimal value, and a contraction elicited at this moment will be larger than the first.

This theory was dismissed by Adrian when he found that the supernormal phase is still present at acid reactions, but is abolished by alkali.

In the paper to which reference has already been made [Dale, 1930], it was suggested that the staircase phenomenon in the rabbit's ventricle was due to a cumulative action of the contractions. In this muscle it is impossible to assume the presence of an optimal interval, for when the rhythm at which a strip of ventricle is being driven is accelerated, the beat following the first short interval is diminished in amplitude, to be followed by an ascending staircase of beats. In the frog's heart, on the other hand, there is an optimal interval, the first beat after a change from a halved to a full rhythm being larger than its predecessor. If, however, the optimal interval were the only factor involved, we should expect the beats following this enlarged one to equal it in amplitude. Actually there is a staircase, suggesting that here also there is a cumulative action of the contractions. Again, when the rhythm of the strip of frog's ventricle is halved, the beat after the first long pause is smaller than its predecessor, but instead of the subsequent beats remaining at this amplitude, they show a gradual decrease in a descending staircase. This suggests that the supposed cumulative action of the beats at the full rhythm leaves some after-effect, which passes off gradually, and only when this has disappeared does the amplitude of the contractions at the halved rhythm become constant. Further, if the amplitude of the contractions at any given rhythm is determined by their cumulative action, this action must be more intense at fast rhythms than at slow, and we might expect the after-effect to vary accordingly. A series of experiments was carried out to test this point. Pauses of varying length were interposed in two series of contractions, one at 12 per min. and the other at 6 per min. and the amplitude of the first beat after each pause was taken as a measure of the intensity of the after-effect at that interval.

In order to obviate errors due to friction between the writing point and the drum, the movements of the lever were recorded by throwing its shadow on to a moving sensitive paper. The strip of frog's ventricle was prepared and set up as already described, the arrangement of the lever being slightly different, as the sensitive paper in the camera moved vertically. A small L-shaped piece of brass was pivoted at the angle, the light straw pointer was attached to the vertical arm, and the thread from the muscle to the horizontal arm. The downward movement of the latter was resisted by an elastic band. After setting up the preparation, 2 hours, during which it was rhythmically stimulated, were allowed for it to come into equilibrium with the surrounding Ringer's fluid. It was

found by experience that the amplitude of the contractions gradually increases over such a period, then reaching a constant value. A series of records was then taken, interposing pauses of various lengths in the series of stimulations. As will be seen from Fig. 3, the pauses varied from 2 to 10 min. A few beats were recorded during rhythmical stimulation,

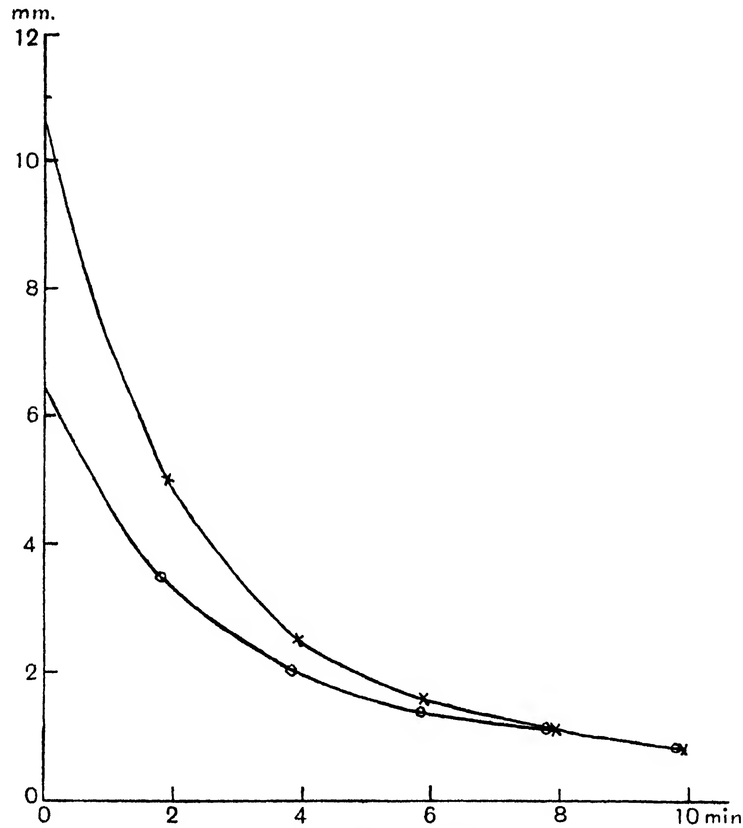

Fig. 3. For description see text.

stimulation was stopped for the desired interval, and then a record made of the first few beats on resumption of stimulation. It is very important, if consistent results are to be obtained, that sufficient time be allowed for recovery after a pause, before the next observation is made. After a pause of 10 min., as much as half an hour may be necessary before the amplitude of the contractions again reaches a constant value, and enables another test of the effect of an interruption to be made.

Fig. 3 shows the results of a typical experiment plotted graphically. The length of the pause is plotted as abscissa, and the corresponding first excursion of the lever in mm. as ordinate. The durations of the pauses plotted represent the excess over the normal interval in the rhythm of stimulation, thus enabling the excursion of the lever characteristic of rhythmic stimulation to be plotted against zero time. It is evident from the figure that the after-effect left by a series of contractions at a rate of 12 per min. is greater than that left by a series at half that rate. Further, the after-effect due to the beats at the faster rhythm disappears more rapidly at first than that produced by those at the slower rhythm, so that after an interval of 8 min. the curves meet and descend together.

In the previous paper, a tentative theory was put forward to explain the fact that, when the rhythm at which a strip of rabbit's ventricle is beating becomes slower, the beat following the first long pause is enlarged. It was suggested that this enlargement might be caused by such an after-effect as has just been described for the frog's ventricle. If this explanation holds, the size of this first beat should decrease as the length of the pause increases, and a series of experiments was therefore carried out on perfused strips of rabbit's ventricle to test this.

The perfusion apparatus was the same as that already described for rabbit's ventricular strips. It is very important for quantitative experiments, however, that conditions such as temperature should be kept constant. The strip preparation, the warming coil, and the tube connecting the two, were therefore enclosed in a box, which was heated by means of a carbon filament lamp, which maintained the temperature inside the box in the neighbourhood of 34° C. The Mariotte's bottle and oxygenating tower were outside, and the tube connecting the latter with the warming coil passed through a hole in the top of the box. The contractions were recorded by means of a Wiggers' miniature myocardiograph [Wiggers, 1916]. This was slightly modified for the present purpose, the needles which form the attachments to the heart muscle being placed further apart, in order to include a greater length of muscle between them, and so give a bigger excursion. This was accomplished by soldering a small piece of brass on to the bottom of the capsule, and mounting the fixed needle on this. The capsule was joined by thick-walled rubber tubing to a piece of lead pipe, which passed through the side of the box, and made connection with the recording capsule outside. A T-tap was placed in this connection, so that the myocardiograph could be connected, either with the recording capsule, or to the side tube of the T-tap which was open to the air. Between the taking of records the tap

was turned so that the myocardiograph was connected to the side-tube, allowing the muscle to shorten, and thus keeping it in better condition. It is important, in recording from this preparation which hangs freely from the cannula, that the myocardiograph should be held fairly rigidly. If it is free to move, the contraction of the strip twists it into such a position that the approximation of the needles is minimal, and a very small excursion is obtained on the record. An optical method of recording was used, the recording capsule carrying a small mirror which reflected the image of a bright line on to the lens of a moving paper camera. The bright line was the filament of a "Baby Ciné" lamp. The stimulating electrodes were two fine silver wires which hooked into the muscle. The

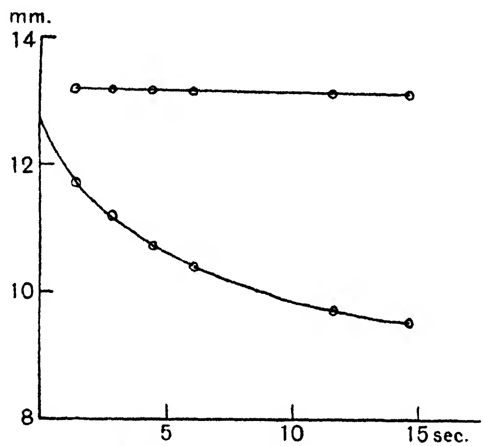

Fig. 4. For description see text.

rotary contact breaker, already described, was used, and a switch was included in the primary circuit, so that a pause could be interposed in a period of rhythmic stimulation by cutting out any desired number of beats. It was found that when the myocardiograph is attached to the strip, the optimal rhythm is much slower than when the strip is pulling on a lever, as in previous experiments. At a rhythm of 40 per min., the muscle remained in good condition, but at rhythms much faster than this fatigue set in. The pauses were relatively much shorter in duration than those employed in the experiments with the frog's ventricle. The minimum pause was that produced by cutting out one beat, while the maximum was determined by the fact that after pauses of a certain length, varying in different preparations, the first beat is abnormal, in that it is followed by a spontaneous premature contraction. In some preparations this may

appear after pauses of five beats' duration, in others not until the pause has reached a duration of fifteen beats. In the preparation from which Fig. 4 was obtained, it was possible to cut out ten beats without the appearance of a premature contraction on return to stimulation.

The excursions of the spot of light on the paper were measured and plotted against the duration of the pauses in the same way as was described for the experiments with the strips of frog's ventricle. Fig. shows the results of a typical experiment. The upper straight line give the values for the enlarged first beat, and it will be seen that the duratic of the pause has no effect on the amplitude of this contraction. Tl amplitude is the same, whether the pause be of one or ten beats' duratio The explanation of the enlargement of the first beat put forward in t previous paper is therefore untenable. If, however, the excursion of t spot of light for the beat following the first enlarged one is measured, is found that this decreases as the pause increases in duration, and t lower curve of Fig. 4 is obtained.

Discussion.

The experiments just described strongly support the theory that staircase in cardiac muscle is due to a cumulative action of the (tractions. The results obtained give little evidence, however, as to nature of this action. There are two possibilities: (1) as suggestec previous writers, it may be of a chemical nature, an accumulatio some metabolic product which favours muscular contraction; (2) a ch in the visco-elastic properties of the muscle brought about by the tractions.

The first possibility is supported by the experiments of Joffé [on the ventricle of the frog. He finds that fluid which has perfu rhythmically stimulated, isolated ventricle, augments the contra of a second ventricle when added to the fluid perfusing it. The ac of the fluid is greater, the larger the number of contractions perf by the first ventricle, while the fluid is in its cavity. If such a subs is the responsible factor, the production of the staircase may be exp as follows. During the ascent of the staircase the active sub diffuses away less rapidly than it is formed, so that its concentrat the muscle increases and favours an increase in the amplitude contractions. As the concentration increases the rate of diffusi increase, and a point will be reached when the rate of diffusion equ rate of formation. The concentration of the substance, and likew

amplitude of the contractions, will then have reached a maximum, and will remain constant. Further, if we assume that every contraction liberates an equal amount of this substance, the faster the rhythm, the greater will be the rate at which the substance accumulates. At fast rhythms therefore a high concentration must be attained before the rates of formation and diffusion are equal, and the resultant amplitude of contraction will be large. Again, in considering the curves of Fig. 3, it was pointed out that the curve obtained when the initial rate was 12 per min., fell more steeply at first, than that resulting from an initial rate of 6 per min. This is readily explained, if the initial concentration of the substance is greater at the fast rhythm. Its rate of diffusion will then be greater, and it will disappear more rapidly. In the case of the descending staircase produced by slowing the rhythm, the rate of formation of the substance by the contractions at the slower rhythm will at first be less than its rate of diffusion, the initial concentration being that characteristic of the faster rhythm. The concentration will decrease, however, and with it the rate of diffusion, so that a point will be reached when the rates of formation and diffusion are once more equal. The concentration at this new equilibrium point will be less, and the amplitude of the contractions therefore smaller.

The second possibility, mentioned above, has been suggested by Fischer [1930] to explain the phenomena of the staircase in the sartorius of the frog. He finds that the length of the period of rest preceding the staircase is a more potent factor in reducing the size of the first beat than is the amount of washing that the muscle has received during that period. He concludes that, in this case, the accumulation of metabolites is not the cause of the staircase. As it is not certain that the phenomenon is the same in skeletal as in cardiac muscle, it does not follow that this theory can be applied to the latter.

The first theory fits the experimental facts exactly, as far as the ventricular muscle of the frog is concerned. In the case of the rabbit's heart, it is more difficult to apply. With regard to the question of the supernormal phase, we may assume that, while in the frog's heart enough of the substance is produced by one beat to augment the contraction of a second, in that of the rabbit two beats are necessary before the required concentration is attained. The fact for which the theory has no explanation is the enlarged first beat following a pause. As is shown by the results given in Fig. 4, the amplitude of this beat bears no relation to the length of the pause preceding it, indicating that the substance left by the preceding series of contractions is not responsible. It seems

that some separate factor must be involved. It was thought that this might be connected with the perfusion, but stopping the flow of perfusion fluid during the pause, made no difference to the size of the first beat following it. Further experiments are necessary to determine the nature of this factor, and it is proposed to carry them out on isolated strips of rabbit's auricle. This should be an easier preparation to handle than the ventricle, as it does not require perfusion. That the enlarged beat occurs in the auricle is obvious from the tracings taken from the whole heart, and published in the previous paper (Figs. 2*a* and 3*a*).

Summary.

1. The staircase phenomenon in the perfused ventricular strip of the rabbit is unaffected by changes in concentration of $H^{\cdot}$, $Ca^{\cdot}$, or $K^{\cdot}$ ions in the perfusion fluid.

2. The staircase phenomenon is present in the ventricular strip of the frog. In this preparation it is unaffected by changes in the $H^{\cdot}$ ion concentration of the surrounding fluid, and is not abolished by atropine.

3. The staircase phenomenon in the ventricles of the frog and rabbit is due to the accumulation of some substance liberated during contraction.

In conclusion, I wish to express my thanks to Prof. Barcroft for permission to carry out this work in the laboratory at Cambridge, and to Prof. Adrian and Dr A. N. Drury for their advice and criticism.

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STATE OF THE FLEXOR REFLEX IN PARAPLEGIC DOG AND MONKEY RESPECTIVELY.

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MARKED difference of reflex activity obtains between the paraplegic dog or cat on the one hand and the paraplegic monkey on the other. For instance, the flexor reflex of the hindlimb in the spinal dog and cat is facile and vigorous, in the monkey difficult or impossible to evoke. This diversity is important because evidently correlated with the relative distances respectively separating these two mammalian types from that of man. We have sought to find numerical expression for this, and thus to get a numerical grading for the difference of spinal condition which it represents.

METHOD.

The upper limit of the share of a particular muscle which a given excitatory reflex has at command can for some muscles and some reflexes be ascertained fairly closely by the isometric myograph. That upper limit, peripheral conditions afferent and efferent remaining equal, can serve as test for the central effectiveness of the reflex. To such a test the flexor reflex, sampled by the main ankle flexor, tibialis anticus, lends itself well. It is a reflex in which frequency of reflex discharge tends to correspond with frequency of the sensory stimulation of the afferent nerve; hence the muscle can be reflexly driven at the same rate as the motor tetanus against which it is to be calibrated.

The tibialis anticus is "isolated" for the myograph by putting out of action by nerve section or tendon resection all other muscles of the limb. The limb is fixed by steel drills through upper and lower ends of the tibia and clamped to the table below the myograph. The myograph used was of the torsion bar type fitted with a mirror for registering optically. In one experiment the torsion element was a steel strip [Hartree and Hill, 1921], in the others it was a torsion wire with a rocking knife-edge bearing [Eccles and Sherrington, 1930], with the mirror axially set on the free end of the torsion element. The afferent nerve was the ipsilateral popliteal in the thigh; the motor nerve was the peroneal, all branches except that to tibialis being cut distally. Observations were made on the dog, cat and common macaque monkey. In the former the cord was in every case cut between 9th and 10th thoracic segments: in one monkey the cord was at 11th thoracic, in the other three at 8th thoracic

segment. Where the myographic examination was made within a few hours of the severance of the spinal cord, the procedure was, before relaxing the anæsthesia employed during the operation on the cord, to decerebrate, and then forthwith to discontinue the anæsthetic. Then 3–4 hours later the myographic examination was undertaken. Records were made of (1) the reflex at its strongest, (2) of the maximal motor tetanus. The samples of these were usually taken alternately, the motor nerve being stimulated in its continuity.

Results.

The experiments fall into two groups: (1) where the myographic examination was made 3–4 hours after spinal transection; (2) where it was made not earlier than the 21st day after the transection. These latter are marked *c* (*i.e.* "chronic") in the accompanying table.

TABLE. Contraction tension in g.

	Wt. in kg.	Reflex	Motor	Reflex as p.c. of motor
Dog	7·5	3490	4330	82
,, *c*	6·3	3250	3660	88
Cat	—	2510	3150	80
,,	—	1750	2220	79
,, *c*	2·1	1800	2140	84
Monkey	3·6	0	3920	0
,,	3·2	? 10	3450	0
,,	1·8	0	3950	0
,, *c*	2·5	95	210	46

Where the observations were taken within a few hours after cutting the cord the value obtained for the reflex both in dog and cat agreed fairly with results observed in the cat and recorded in a former paper [Cooper, Denny-Brown and Sherrington, 1926]. There the value was 77 averaged from 4 cats; here 79·5 for the cats and 82 for the dog. Against those figures two of the three monkeys gave 0, and the third less than 0·5, an extreme contrast.

(1) Evidently in the early "acute spinal" state the spinal activity of the mammalian type less remote from man is, as judged by this reflex, vastly inferior to that of the type more remote from man. The extensor reflexes it is true are in the "acute spinal" dog and cat much more depressed [Sherrington, 1910] than is the flexor reflex which is freed from prespinal inhibition [Sherrington and Sowton, 1915]. With the extensor reflexes therefore the inequality between dog and monkey is hardly so marked, although with them also the reflex inactivity of the early "acute spinal" state is greater in the monkey than in the dog and cat, witness the frequent total abeyance of the knee jerk even for days in the "acute spinal" monkey.

(2) The reflex activity of the isolated cord tends to show some pro-

gressive increase for periods covering days and weeks and even months. Our observations were therefore extended to see how far the great difference obtaining between dog (and cat) and monkey in the "acute" experiments would hold also over longer periods. The myographic examination was conducted subsequent to complete healing of the operation wound, the spinal severance having been performed with full precautions for asepsis. In each case the general health of the animal was, apart from the condition of paraplegia, good.

Dog, small; 7. xii. 29; 10th thoracic spinal segment excised. Wound healed rapidly. Animal remained in good health. Usual reflex activity; stepping of hindlimbs on being raised; bilateral active extension of hindlimbs on first allowing the limbs to hang pendent. 2. vi. 30. Weight 6·3 kg.; anæsthetized and decerebrated at 10 a.m.; limb prepared, and myographed at 2.30 p.m. Results see Table.

Cat, young; 24. iv. 30, cord cut at 10th thoracic. 1. viii. 30. The wound had healed quickly and the general health has remained good, and animal has grown; weighs 2·1 kg. Reflexes; spring clip to hindfoot excites quick and prolonged flexion reflex, with some stepping of opposite hindlimb. The flexion reflex under persistence of the stimulus tends to break into irregular alternating movements including adduction-abduction of limb, as if to liberate the limb from the stimulus. Light touching of the toepads or plantar cushions evokes lifting and spreading of the toes. When spinal stepping is in progress light passive support of one thigh in its descent toward extension stops the stepping in both limbs. At 10 a.m. decerebrated under anæsthetic. Limb prepared: myographic examination at 3 p.m. Results see Table.

Monkey, *Macacus rhesus*; 2. vii. 30, forenoon, cord transected at 8th thoracic segment, under dial. 9. vii. 30. Wound has healed well: feeds well and has done so since late afternoon of 3rd. Complete flaccid paraplegia of hindlimbs; no reflexes; slight decubitus ulcer on right hip. 14. vii. 30. Feeding well, and is lively. No knee jerk; adductor response to tapping tendon; slight flexion of hallux on pinching toes. Some ulceration on right hip; extensive wasting of muscles; no trouble with bladder; rectal temp. 37°C. 19. vii. 30. Wasting of hindlimbs more marked; knees and ankles stand out like knobs in the flaccid limbs. Belly wall relaxed; no knee jerks, but adductor jerks present. Flexion of hallux on pinching foot, accompanied by slight flexion of ankle. Feeds well; is very active with forelimbs; ulcer still present over right hip; temp. normal. Photographed.

22. vii. 30. Good general condition apart from the paraplegia; no knee jerks; adductor responses on tapping tendon; flexion of hallux on pinching foot. The small sore over trochanter is covered with clean granulation tissue and has not led to general infection; no induration of surrounding muscle. Wasting of hindlimb muscles is extreme. Afternoon: myographed. As the animal showed no sensation whatever at any point below 8th thoracic level no general anæsthetic was used: an assistant merely nursed and amused the animal while the hindlimb (left) was prepared. Tetanization of central ipsilateral popliteal nerve gave sluggish retraction of the cut tib. ant. tendon which lay free; also evident contraction of the adductor of opposite thigh. In the myograph the threshold for tib. ant. reflex from popliteal was 11·5 cm. (coreless coil) and at 10 cm. had reached its maximal effect, *i.e.* 95 gm. contraction tension. Tetanic stimulation of uncut peroneal nerve (its branches except that to tibialis anticus having been cut peripherally) gave threshold for motor response at 15·5 cm. and was maximal at 12·5 cm. giving 210g. contraction tension. The motor and reflex responses were taken approximately alternately. The tetanic contractions

relaxed very sluggishly, the "motor" tetani quite as slowly as the "reflex." The "latent period" of the reflexes was always very long, *i.e.* between 80σ and 90σ. To test possible escape of current for reflex stimulus at 10 cm. the electrodes remaining as placed on popliteal the peroneal was then cut proximal to the anatomical meeting point of the two nerves; the response from popliteal then entirely disappeared; it was therefore wholly reflex. Single break shock stimuli then applied to distal peroneal found motor threshold at 15·9 cm. and maximal at 12·5; the maximal twitch reached 100 g. tension. The twitches like the tetani showed quite abnormally slow time relations (*v. infra*).

Necropsy showed complete and clean spinal severance at 8th thoracic; the opposite face of the cut cord were 3 mm. apart, with some young scar tissue between them. The cord appeared healthy to naked eye except for obvious "tract" degeneration detectible to close inspection above and below the lesion.

Discussion.

The myograph showed the flexor reflex both in dog and cat to have a distinctly higher value in the "chronic" spinal condition than in the "acute," 88 and 82 as against 80 or a little less. It was not clear from the few experiments that the power of the "motor" tetanus itself falls in those animals in the "chronic" spinal condition necessarily far below normal; but in the "chronic" monkey a peripheral change was met with, impairing the nerve-muscle complex itself. This peripheral impairment was a complication for the evaluation of the reflex. The occurrence of muscular wasting even of dystrophic character in the paraplegic limbs had not been unexpected [Sherrington, 1898; McCouch, 1924]. McCouch in his observations in the monkey found microscopic changes (advanced chromatolysis) in the ventral-horn cells of the spinal grey matter of the limb region accompanying a severe paraplegic state of 35 days' duration. As to the human cord, Dr Gordon Holmes writes us from first-hand experience that he has repeatedly observed chromatolysis in the motor-horn cells below severe transverse lesions. We found in our 3 weeks' monkey the contraction responses of the flexor muscle under direct stimulation of its motor nerve to be in fact severely defective and abnormal [cf. also Matthes and Ruch, 1931]. The maximal twitch of tibialis anticus for a monkey of the kind, age and weight we were using should, to judge from the other three monkeys of the series, be about 900 g., whereas the value found (Fig. 1) was 105 g., nearly a 90 p.c. deficit. The ratio twitch tension/tetanic tension proved abnormally high 45/100, instead of about 28/100 as would be expected. For the motor tetanus the subnormality of tension was therefore even greater than for the twitch. The enfeeblement of the response and the wasting of the muscle were more than mere disuse could account for. Moreover the time relations of the contractions were grossly abnormal. The twitch had, following the nomenclature of

Cooper and Eccles [1930], a "contraction time" of 140σ, *i.e.* took that time to reach its maximal tension; and the whole twitch lasted some 900σ. The maximal tetanus took nearly a second to relax. In *Macacus rhesus* normally this muscle has a contraction time of barely 30σ, and reaches complete relaxation in about 210σ (Fig. 1). The weight of the tibialis anticus muscle in the paraplegic monkey of 3 weeks' standing was 2·8 g., whereas in a normal *Macacus rhesus* of rather lighter body weight the tibialis anticus weighed 5·5 g. Miss Cooper kindly compared the maximal twitch tension of tibialis anticus of a normal *Macacus rhesus* (1·8 kg. body weight), the muscle with its tendon weighing 5·6 g., with that of a

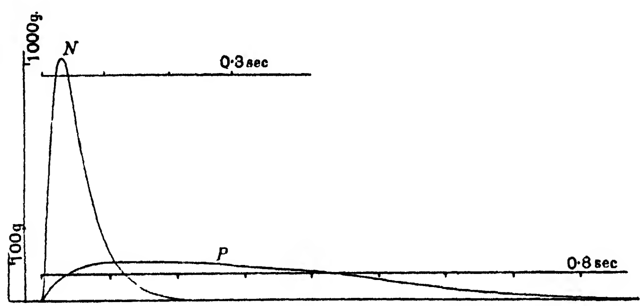

Fig. 1. Isometric records of maximal motor twitch of tibialis anticus of monkey *P*, *M. rhesus*; 2·4 kg. body weight; 3 weeks' paraplegic; *N*, *M. rhesus*; 1·8 kg. body weight; normal. Separate tension scales for *P* and *N* to left; times as abscissæ.

tibialis anticus weighing 4·8 g. from a 2·4 kg. cat. The monkey's tibialis gave for maximal twitch 1040 g. tension, the cat's 720.

Our monkey's reflex had therefore a defective nerve muscle to take expression through. If that be considered not to invalidate the application to this case of the test used in the others for evaluation of the amount of reflex, then the reflex in this case can be graded at 45. In support of accepting the figure, as an approximation, is the circumstance that both elements of the ratio, the "reflex" and the "motor" are measured through the same deteriorated nerve-muscle preparation. The importance of the value thus obtained is that although much smaller than in dog (and cat) it is nevertheless much larger than that obtained in the monkey in the "acute" spinal state. This argues that there does exist in the hindlimb of the monkey a spinal flexor reflex, although immediately subsequent to hind thoracic severance of the cord it is so enfeebled as to be not then obtainable. That there is some degree of variability from experiment to experiment in regard to this enfeeblement is likely; our two observations indicate that. The spinal knee jerk is similarly variable. In

the second and third monkeys of our series the spinal transection, at 8th thoracic level, was not followed by disappearance of the knee jerk. The knee jerk was feeble but remained present even from immediately after the performance of the transection. This is unusual [Fulton, 1932; Sherrington, 1898]; and in the first and fourth monkeys of our series, although of the same species and age, the knee jerk was absent after the transection, and in the fourth animal did not return during the subsequent 3 weeks.

SUMMARY.

1. In the monkey (*Macacus rhesus*) after spinal transection in the thoracic region the flexor reflex of the hindlimb instead of being facile and vigorous as in the dog (and cat) is for a time unobtainable or if obtainable is so merely as a trace.

2. In the course of some weeks in monkey, as in dog (and cat), there ensues some increase of the reflex, the reflex in the monkey can become distinctly elicitable, but in the monkey there supervenes a peripheral condition of wasting and deterioration of the nerve-muscle response ("isolation dystrophy"), which tends to obscure observation of such central reflex action as exists by impairing its means of expression.

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THE RÔLE OF THE PITUITARY GLAND IN PREGNANCY AND PARTURITION.

I. Hypophysectomy.

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Technique.

It has been stated frequently that the invariable result of hypophysectomy during pregnancy is immediate abortion, yet the amount of experimental work which supports this conclusion is small. A series of hypophysectomies was therefore performed in pregnant animals to test the truth of this statement. Obviously, the operation must be done in such a way that no structure but the pituitary is injured, and that removal is complete. There are available a number of methods of approaching the pituitary. Amongst these are the nasal, the trans-dural, the buccal and the retro-pharyngeal; the first two are not convenient in the cat, and we have used only the two latter as fulfilling as nearly as possible the necessary conditions. That is to say, the removal is effected in full view of the operator, and at the same time the risk of damage to adjacent structures is reduced to a minimum.

One of us favours the buccal route, and the other the retro-pharyngeal. The buccal route permits of a more deliberate operation and gives, perhaps, the best view of the pituitary region, but it has the serious drawback that it is particularly liable to be followed by a severe nasal infection which may spread down to the lungs with fatal result. In any case, the post-operative period requires special care and skilled and conscientious nursing. With the retro-pharyngeal route these drawbacks are to some extent overcome, but the operation has to be conducted with considerable speed as retraction of the trachea cannot be indefinitely prolonged; the necessary retraction, moreover, somewhat interferes with the space available for manipulation.

It is not proposed to deal in this paper with the results in detail, or the full conclusions to be drawn from them. A new operative procedure is described, and some modifications of an operation already used by McLean.

I. The retro-pharyngeal route.

This will be described in detail, as it is, in many respects, a new one. Access is obtained by dissection of the neck, the pharynx is stripped from the base of the skull and the bone drilled over the pituitary fossa without exposure of any septic surfaces.

Anæsthetic. We have found an ordinary ether induction followed by ether and air administered by a pump through an intra-tracheal catheter to be the best method. The intra-tracheal catheter is essential to maintain an airway when the trachea is retracted. Experiments were made with various non-inhalation anæsthetics, but with all of them there is the common disadvantage that narcosis is prolonged and the return of the cough reflex delayed; this results in stagnation of secretions in the pharynx and a liability to infection which otherwise is avoided.

Pre-operative care. Only animals which have been some weeks in the establishment are used—a point which greatly assists convalescence. The day before operation only milk is given and on the morning of the operation nothing but water. Both at the beginning and at the end of operation the mouth and pharynx are thoroughly cleansed and swabbed with a 1 p.c. solution of mercurochrome. The skin of the front of the neck is shaved and purified by swabbing successively with 5 p.c. phenol solution, alcohol and iodine. A full surgical aseptic technique is used throughout.

Operation. The animal is tied out in the dorsal position and the chin well extended with a weighted hook. A skin incision about 3 in. long is made in the mid line of the neck, extending from ½ in. below the jaw to the sternum. Skin flaps are dissected back, and towels fastened to skin edges with Michel's clips. The transverse vein under the chin is divided between ligatures, and the sterno-mastoid muscles are separated with the aid of a knife; this exposes the infra-hyoid muscles which are separated by blunt dissection from the carotid structures. The nerve to the infra-hyoids is isolated but not cut, but the nerve to the thyro-hyoid group has usually to be sacrificed. The hypoglossal and external laryngeal nerves are seen at the top of the wound but are not in any serious danger. The trachea and œsophagus are lifted up from the pre-vertebral fascia and held aside by a retractor, great care being taken not to compress the trachea so as to interfere with the air return. The pharynx is similarly separated for a short distance until the median fascial septum comes into view. This is then picked up with forceps and the fascia overlying the pre-tracheal muscles is incised just posterior to it. The separation proceeds

cranially, dorsal to this plane which eventually becomes continuous with the muco-periosteum covering the base of the skull. This muco-periosteum is separated from bone by swab dissection to just beyond the tip of the pterygoid bone, which can readily be felt with the finger; this is about $\frac{1}{4}$ in. beyond the large bullæ which lie immediately laterally to the pharynx. An occasional touch with a sharp-pointed knife may be necessary at the extreme lateral margins and care must be taken to avoid several small veins in this region.

The pharynx is now retracted by a specially designed retractor; this is about $\frac{1}{2}$ in. wide and curved on the flat so as to fit round the hole to be made in the bone. When using it, it is imperative to avoid bruising the thin wall of the pharynx. The point selected to drill the bone is almost exactly opposite the pterygoid and not infrequently a small emissary vein may be seen issuing at the precise spot. We have been in the habit of using an ordinary dental burr driven by a foot-drill to pierce the bone, the hole being enlarged with the largest-sized burr in very much the same manner as a cavity in a tooth. Injury to the dura is easily avoided, and with a little trouble an opening at least as large as the pituitary fossa can be obtained. The cavernous sinuses lie one on each side of the pituitary fossa and joining them posteriorly is a large communicating sinus; the anterior side is comparatively free from blood vessels, so it is better to err in an anterior direction when drilling the bone.

The dura is incised at the central point between the sinuses, and the pituitary immediately bulges through it. A large-sized sucker attached to a water pump is applied, and a considerable portion of the pituitary thus removed; escape of cerebro-spinal fluid and some bleeding follows. The bleeding is controlled by application of crushed muscle. The portions of the pituitary lying circumferentially under the dura escape removal with the sucker, and are sought for with a small, curved, blunt-edged spoon. The pituitary is not shut off from the general cranial cavity by a "diaphragm" of dura as in some animals, so injury to the infundibulum cannot always be avoided. Should one of the large sinuses be injured the bleeding can usually be controlled in a few minutes by packing over a piece of crushed muscle; once or twice, however, it has been necessary to leave in a small muscle plug. The hole in the bone is filled with Horsley's wax, the superficial muscles are brought together with a running suture and the skin wound closed with interrupted stitches.

After-care. The wound is painted with Whitehead's varnish, but no dressing is applied. The cat is placed in a warmed cage on a bedding of cotton-wool—we have found this last point of no small importance in

obtaining healing by first intention which now almost invariably occurs. The stitches are removed after about a week.

Water is allowed immediately the animal comes round from the anæsthetic, but for the following 24 hours nothing else is permitted; thereafter milk is given, and at the end of 48 hours raw meat is usually taken. A sardine or other tasty morsel may assist in re-establishing the appetite. When this route is employed there is seldom any difficulty from sepsis of the pharynx or elsewhere, we have lost but few cats from this cause, and there is very little trouble in the post-operative period.

II. The buccal route.

Full details of the operative procedure in the removal of the pituitary by the buccal approach are given by McLean [1928]. The operation is performed with the cat on its back, the mouth held widely open; the soft palate is divided for about 2 cm., the muco-periosteum over the sphenoid is incised and retracted, and a hole drilled in the bone. The pituitary is seen at the bottom of the hole and is removed by suction under direct vision.

The modifications we have adopted are:

(1) *Anæsthetic.* Nembutal 844 has proved very satisfactory, as, in doses of 0·04 g. per kg., it gives adequate relaxation and the animal recovers consciousness in about 12 hours. It is given by intra-peritoneal injection as a 5 p.c. solution.

(2) *Nasal sepsis.* McLean's paper is mainly concerned with dogs, in which apparently nasal sepsis is infrequent. In the cat, however, nasal sepsis is only too easy to set up, and almost impossible to cure. We have, therefore, taken extreme precautions to avoid the entry of blood or mucus to the nose from the pharynx. For this purpose the posterior edge of the soft palate is pulled forward and a pledget of wool passed forward behind it, so that the posterior nares are plugged before the soft palate is incised. Further, to obtain a clear field and prevent the oozing of blood from the cut edges of the palate, we now use a solution of adrenaline 0·1 p.c. and 10 p.c. cocaine for local application to both surfaces of the soft palate before it is divided. The same solution is used to diminish the somewhat free bleeding from the muco-periosteum covering the sphenoid.

(3) *Whitehead's varnish.* This has been used to paint over the sutures (catgut 000) in the muco-periosteum and in the palate. We believe it gives better healing in what must be, whatever precautions are taken, a relatively septic cavity.

The actual removal of the pituitary, after it has been exposed through the mouth, is the same as that described above for the retro-pharyngeal route.

Results.

The earlier operations were followed in many cases by the delivery of normal-looking fœtuses in 2–3 days. It was at that time impossible to obtain pregnant cats whose date of impregnation was known, so that there was no evidence that the delivery was not occurring at the appropriate time. The greatest time which we have so far observed to elapse after operation before delivery occurred is 11 days. In only one of the series did the mother make any attempt to suckle her kittens, and in this case she abandoned the effort after 2 days; in all the other cases the mother ignored the kittens. Serial sections were made, in a number of cases, of the infundibular region of the base of the brain, and of the dura lining the pituitary fossa, to prove that the removal had been complete; details will be published later.

Table I.

	Lateral pharyngeal				Buccal		
No.	Delivery or laparotomy	Days after operation	Fœtus alive or dead	No.	Delivery or laparotomy	Days after operation	Fœtus alive or dead
33	Laparotomy	10	1 A.	32	Delivery	3	1 A., 2 D.
36	Delivery	2	3 A.	35	Delivery	4	1 A.
42	Delivery	11	3 A.	43	Delivery	4	1 A., 2 D.
45	Laparotomy	5	3 A.	44	Delivery	5	4 A.
52	Laparotomy	5	5 A.	46	Delivery	8	1 D.*
56	Delivery	5	Fœtuses eaten	54	Delivery	2	3 D.*

* Premature.

Further experiments are in progress to ascertain whether the pituitary is essential to the continuance of pregnancy even during the earliest stages.

Conclusions.

1. Delivery can take place, apparently quite normally, in the absence of the whole pituitary.
2. Delivery does not necessarily follow immediately on removal of the pituitary.
3. Suckling has not been observed to take place.

This work has been carried out in the Courtauld Institute of Biochemistry by the courtesy of Prof. E. C. Dodds, to whom we are greatly indebted for his criticism and advice. One of us (H. A.) was working under a grant from Dr R. A. Gibbons, donated for the purpose of investigating the cause of the onset of labour.

We wish to express our thanks to Mr E. R. E. Spence, our laboratory assistant, for his technical help in the work and the care he has devoted to the animals.

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THE INFLUENCE OF INSULIN ON THE LIVER GLYCOGEN OF THE COMMON GREY AUSTRALIAN "OPOSSUM" (*TRICHOSURUS*).

By B. CORKILL.

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THERE is now a body of evidence clearly indicating that, in respect of liver glycogen, insulin produces two sharply contrasted effects, when administered to different normal species. In young rabbits and kittens, for example, a definite glycogen storage has been demonstrated [Goldblatt, 1929, 1930], while in mice, rats or chickens no such storage occurs [Cori and Cori, 1928; Corkill, 1930]; in fact, the predominant effect is that of depletion. Dale and his co-workers have already advanced an explanation for the latter type of effect, whilst more recently [Corkill, 1930] evidence has been furnished to suggest that the glycogen storage observed when insulin is administered to young rabbits is not directly due to insulin but requires the cooperation of adrenaline, secreted in response to the insulin hypoglycæmia. Perhaps mice afford the most striking example of a species in which insulin causes an actual disappearance of liver glycogen, whilst young rabbits afford an excellent contrast, since here the animals injected with insulin often show liver glycogen values seven to ten times greater than the controls. It might be objected that, in view of the much more rapid metabolism of mice, it is not legitimate to compare such animals with rabbits; and it is chiefly from this aspect that the results obtained with the adult opossum are of interest. The opossum, in rate of metabolism, corresponds to the rabbit; but the values observed for liver glycogen, after the injection of insulin, are so strikingly different from those found in young rabbits that a brief description should prove of interest.

Attention was first directed to the opossum by the suggestion that in these animals the suprarenal glands were, in comparison with the lowest mammal, *i.e.* the platypus, in a state of retrogression. According to MacKenzie [1921], "in the koala and opossum the suprarenals would appear from their structure and small size, as compared, for example, with the platypus, to play a not very essential part in their economy. In

fact, the right suprarenal gland is frequently absent and grades in its disappearance may be demonstrated." These statements suggested the desirability of investigating the action of insulin in such animals; but, in the twenty animals which formed the basis of the following investigation, the right suprarenal gland was invariably present, although in many instances placed under the hepatic capsule and, therefore, in close relationship to the liver. Histologically, no departure from the normal has been observed, and certainly no cortical invasion by lymphocytes. In several instances suprarenal extracts prepared from right and left glands with acidulated saline gave practically identical results when tested by the spinal-cat blood-pressure method. Finally, the suprarenal glands from the opossum compare, in respect of size, quite favourably with those, say, from an adult cat of the same weight.

Experimental methods.

The name "opossum" applied to these animals is in a sense a misnomer, since they differ widely in habits from the American Didelphidæ, or true opossums. These latter animals are carnivorous, whilst the Australian opossums belong to the eucalyptus-leaf eaters.

In the present experiments adult opossums weighing from 1·5 to 1·8 kg. were used. In the Australian opossum single births are the rule, in marked contrast to the true opossum, which will sometimes be seen carrying a dozen young ones on its back. Hence it was impossible to conduct experiments on similar lines to those with young rabbits, in which case the animals receiving insulin were compared with "controls" from the same litter. Accordingly the plan adopted was to select opossums of approximately the same weights for "control" and "insulin" groups. The adult opossum displays quite a definite resistance to insulin, since 10 units are usually required to produce a definitely hypoglycæmic state. The insulin (Commonwealth brand) was injected subcutaneously over the thigh muscles, and on the development of flaccidity and incoordination (usually within 3–4 hours) the animal was killed by a blow on the head. The thorax and abdomen were then quickly opened, the liver weighed and dropped into tubes of boiling 60 p.c. KOH. The glycogen determination then followed the procedure described in a previous paper [Corkill, 1930]. In addition, blood was taken from the heart and glucose estimated according to the method of Hagedorn and Jensen.

Results.

The results are presented in two groups. In the first series the experiments were conducted after a period of 24 hours' fasting, whilst in the second series the period was extended to 36 hours. In all instances insulin failed to produce a deposition of liver glycogen.

The "control" figures, particularly those of the second series of experiments, afford a very favourable basis on which to interpret the insulin figures, which certainly do not indicate glycogen storage in the liver. The results of these two series of experiments are shown in Tables I and II.

Experiment.

Six adult opossums were taken and kept without food for 24 hours. Three were then set aside for control purposes, whilst the remainder were injected with 10 units of insulin. The values obtained for liver glycogen are shown in Table I.

Table I. *Adult opossums fasting for 24 hours.*

	Controls			After insulin (10 units)		
	Weight of opossum g.	Liver glycogen p.c.	Blood sugar p.c.	Weight of opossum g.	Liver glycogen p.c.	Blood sugar p.c.
	1502	1·8	0·124	1520	0·45	0·052
	1345	0·95	0·115	1300	0·48	0·050
	1425	1·12	0·128	1250	0·75	0·045
	1650	1·05	0·110	1620	0·60	0·040
Average	1480	1·23		1423	0·57	

In the next series of experiments the opossums were kept without food for 36 hours. The figures shown in Table II represent several experiments, but, since the experimental conditions were identical, the results may be grouped together.

Table II. *Opossums fasting for 36 hours.*

	Controls			After insulin (10 units)		
	Weight of opossum g.	Liver glycogen p.c.	Blood sugar p.c.	Weight of opossum g.	Liver glycogen p.c.	Blood sugar p.c.
	1725	0·76	0·130	1650	0·65	0·032
	1450	0·51	0·112	1575	0·28	0·025
	1800	0·48	0·105	1260	0·10	0·045
	1685	0·34	0·121	1485	0·26	0·050
	1560	0·38	0·116	1520	0·18	0·030
	1650	0·45	0·108	1616	0·30	0·028
Average	1645	0·49		1518	0·30	

Summary.

In the adult Australian opossum (*Trichosurus*), insulin, in doses sufficient to produce a pronounced hypoglycæmia, without convulsions, causes no deposition, but rather some depletion of liver glycogen.

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612.59:612.13

THE INFLUENCE OF TEMPERATURE IN THE FROG.

(1) On the circulation, and (2) On the circulatory effects of adrenaline and of sodium nitrite.

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(London (R.F.H.) School of Medicine for Women.)

WITH a view to testing the effects of temperature on the action of histamine upon the circulatory system of the frog, a series of experiments was undertaken. This consisted of perfusion experiments, in which the blood-pressure variations were recorded from the aorta, and the changes in calibre of web vessels were studied, either by direct measurement or by observing the flow through them of solutions containing dyes or suspended particles. As a result of these investigations it became clear that, before the effects of change of temperature on the action of a drug could be determined, it was necessary to study the effects on the system of temperature variations alone. Also that satisfactory observations could only be expected when working as far as possible with an intact circulation. A preliminary series of experiments was then undertaken to determine the relationship—if any—between temperature on the one hand, and blood-pressure, heart rate and web circulation on the other.

METHOD.

Spinal frogs were used. The blood-pressure was recorded by means of a water manometer connected with the right arch of the aorta; the heart rate was counted directly through the small incision; and the web circulation observed in the foot attached to a microscope stage. It was found that the natural circulation through the web was not interfered with by the manipulative procedure, provided that bleeding was avoided and that the blood-pressure cannula caused no blocking of the left arch of the aorta. The temperature of the frog was controlled by water dripping onto a cotton-wool jacket surrounding the preparation. The temperature was recorded from a small thermometer inserted into the thoracico-abdominal cavity through the incision.

Results.

Figs. 1 and 2 show the results, given in a condensed form, of two such experiments. A brief analysis of the results obtained from twenty-two experiments in which the temperature was the variable factor showed the ollowing:

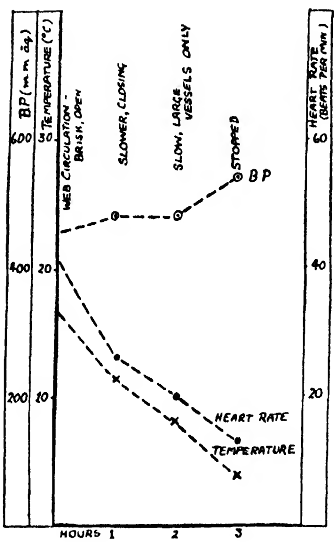

Fig. 1.

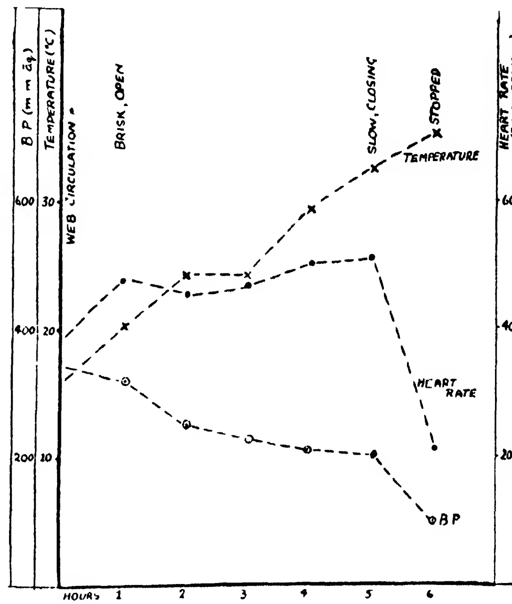

Fig. 2.

Fig. 1. Effects of fall of temperature on blood-pressure, heart rate and web circulation.

Fig. 2. Effects of rise of temperature on blood-pressure, heart rate and web circulation.

1. Confirmatory to the results obtained by Taylor [1930], the relationship between temperature and heart rate was found to be a definitely linear one, except at high temperature (25° C. and upwards).

2. The blood-pressure readings were variable both at constant and at changing temperatures, and there was difficulty in establishing any definite relationship between temperature and blood-pressure. Over prolonged periods, however, cold tended to arrest or recover a falling blood-pressure, whereas heat accelerated a fall. Further investigation of this relationship is at present being made, making use of a subsequent improvement of technique.

3. The web circulation varied chiefly in rate of flow and in the number rather than in the calibre of active vessels. Under the conditions of these experiments the blood-pressure appeared to be a more important determining factor in control of the web circulation than the temperature.

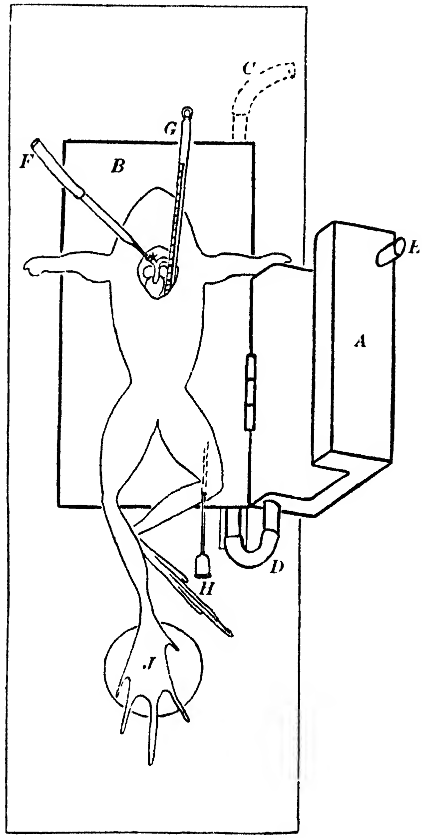

Fig. 3. Copper-box apparatus, with outlined position of frog. *A*, open lid; *B*, base inset into cork; *C*, inlet for water (under cork); *D*, communication between base and lid; *E*, outlet for water; *F*, cannula in aorta for blood-pressure; *G*, thermometer; *H*, syringe needle in lymph sac; *J*, web prepared for microscope stage.

Changes of temperature between 10 and 20° C., unless accompanied by some gross change in the blood-pressure, produced very slight variations, if any. Between 20 and 25° C. the web circulation often appeared to be brisker and more open (using "open" in reference to the number, not the calibre, of active vessels). Above 25° C. it became sluggish and closed

(*i.e.* number of active vessels diminished), coinciding with the general failure of circulation due to the breakdown of the temperature and heart-rate relationship and the consequent fall of blood-pressure. Below 10° C. a typical "cold effect" was frequently observed. The web circulation was sluggish and the field closed, with marked congestion and pulsation in the active vessels. This "cold effect" developed even when the blood-pressure was well maintained, and, unlike the circulatory failure produced at high temperatures, it was not a death change, but one from which the animal could be completely recovered by warmth.

With the object of controlling the influence of temperature on the circulation more carefully, the experimental method was modified by the introduction into the apparatus of a copper box, within which the frog was enclosed. Through the hollow base and lid of the box flowed a stream of water kept at constant temperature, or varied as required[1] (Fig. 3).

The apparatus was tested by repeating the preceding experiments, with confirmatory results. With this modification it was possible to demonstrate the maintenance of a steady temperature, blood-pressure, heart rate and web circulation over periods up to $1\frac{1}{2}$ hours, and the method, therefore, seemed suitable for use in the investigation of effects produced by drugs upon the circulation.

Experimental work upon the effects of drugs.

Control experiments. The introduction of a syringe needle into the lymph space of the thigh, during the course of an experiment, caused an immediate and marked rise in blood-pressure. This rise occurred whether the frog struggled or not. A similar effect was produced by jerking the needle and so pulling on the skin during an injection, or by withdrawing the needle, or merely by stroking the frog's skin. The syringe needle was, therefore, introduced into the lymph space of the thigh during the preparation of the frog, and remained in position throughout the experiment. With this precaution, the careful injection of 0·5 c.c. of saline, during the course of an experiment, produced only a slight and transitory rise in blood-pressure, easily distinguishable from a true drug effect. For example, an injection of 0·5 c.c. of saline produced an immediate rise of 15 mm. āq., whereas a subsequent injection into the same animal of 0·5 c.c. of adrenaline solution, 1 : 100,000, produced a rise in blood-pressure of 240 mm. āq.

The web circulation, as described by Krogh [1921], was observed

[1] We wish to express to Dr Jeanne Eder of Zurich our thanks for suggesting and superintending the making of this apparatus.

to react to various local stimuli such as direct touch, or the removal of a wet pad. When the web was kept moist with water dropped from a pipette, however, no local reaction was observed.

Method. In the subsequent series of experiments the same method as before was employed, with the modification of the copper-box apparatus. The precautions suggested by the control experiments were observed throughout. Drug solutions were injected in doses of 0·5 c.c. into the lymph space of the thigh. Observations of the blood-pressure, heart rate and condition of the web circulation were taken at frequent intervals, varying with the conditions of the experiment but averaging about five readings an hour.

Histamine injections were tried in a few experiments, but the results obtained showed considerable variation. Calcium chloride injections produced unexpectedly little effect, compared with the markedly stimulating effect produced by the local application of calcium chloride solution to a sluggish web circulation. The study of these drugs was, therefore, temporarily abandoned, in favour of an investigation of the effects of temperature on two drugs, adrenaline and sodium nitrite, whose influence upon the vascular system is consistent and marked. The doses which were found convenient, and were used throughout with a few exceptions, were: adrenaline hydrochloride 0·5 c.c. of 1:100,000 sol.: sodium nitrite 0·5 c.c. of 2 p.c. sol.

Results obtained with adrenaline.

Figs. 4 and 5 show characteristic results in experiments in which adrenaline was injected at low and high temperature. The following results were obtained from 18 such experiments, 4 at 10° C., 6 at 15° C. and 8 at 20° C. (approx.).

The blood-pressure was raised in every case by adrenaline, on an average by 45 p.c. The average time taken to reach maximum pressure was 35 min. at low, 16 min. at medium and 8 min. at high temperature; and the average duration of the blood-pressure effect was 2 hours at low, 1 hour 30 min. at medium and 40 min. at high temperature.

The heart rate was accelerated except in 3 cases, all at high temperature, in which the rate was slowed.

The web circulation in 6 cases (2 low, 1 medium, 3 high) began and ended brisk and open, but was temporarily less good during the adrenaline rise of blood-pressure, when the field became closed, the flow in the active vessels was slowed, and in some cases the calibre of the vessels was diminished. In 5 cases (1 low, 1 medium, 3 high) the web circulation

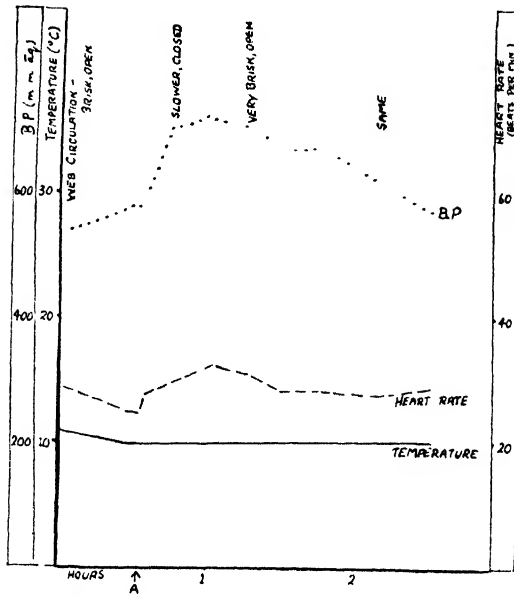

Fig. 4. Effects, at 10° C., of injection, at A, of adrenaline HCl 0·5 c.c., 1:100,000, on blood-pressure, heart rate and web circulation.

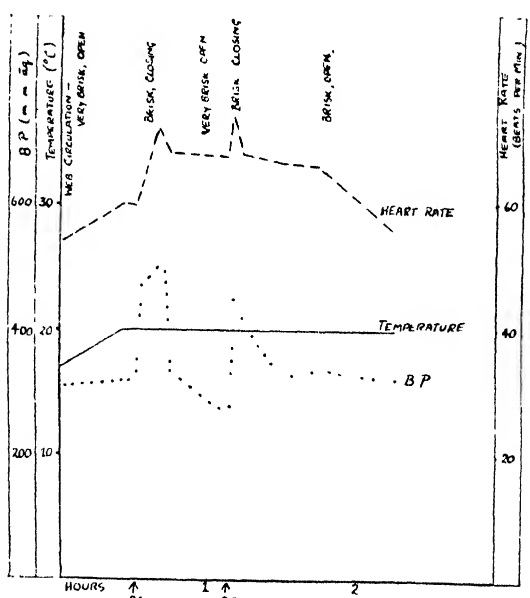

Fig. 5. Effects, at 20° C., of injection, at A_1, repeated at A_2, of adrenaline HCl 0·5 c.c., 1:100,000, on blood-pressure, heart rate and web circulation.

began less than good and in each case was improved by the injection of adrenaline. In 3 other cases the circulation began and remained good throughout, showing no closing during the rise in blood-pressure, which was, however, relatively slight in each case. The remaining 4 cases cannot be classified, as various external factors interfered with the condition of the web circulation during the experiment.

Discussion of adrenaline results.

The percentage rise in blood-pressure caused by adrenaline is similar at high and low temperatures, but the rise develops and passes off more quickly at 20 than at 10° C. This difference (in rate but not in quality of the effect) may, therefore, be related to a difference in rate of absorption of the drug at high and low temperatures, rather than to a modification in the action of the drug itself. Evidence that the former explanation is the more probable was obtained from the results of six experiments in which the frog's vessels were perfused with adrenaline solution at 10 and 20° C. Under these conditions the difference in temperatures produced no detectable difference in the action of the drug.

The failure of the heart to accelerate with adrenaline as consistently at high as at lower temperatures does not necessarily indicate any modified action of the drug. Since the average heart rate is 26 beats per min. at 10° C., 40 at 15° C. and 60 at 20° C., the possibility of transitory sympathetic stimulation failing to produce acceleration seems greater at 20° than at 10° C., even apart from the fact that the duration of the drug effect is shorter at the high temperature.

The type of effect produced by adrenaline on the web circulation seems to bear no direct relationship to the temperature, except that the effects which develop during the rise of blood-pressure are more prolonged at low than at high temperatures. The web circulation is ultimately unimpaired or improved by adrenaline, but the transitory closing of the field during the rise of blood-pressure occurs sufficiently consistently (given an initially brisk and open circulation and a good rise in blood-pressure) to be regarded as a characteristic of the drug. Adrenaline produces a similar but greatly exaggerated effect upon the web circulation when applied directly to the foot, as described by Oinuma [1924] and others. It would seem that the rapid initial rise in blood-pressure is brought about not only by arterial constriction and acceleration of the heart, but also in some cases by a temporary diminution in the number of active vessels in the peripheral circulation, the capillaries of the web acting independently of the arterial pressure, as described by Krogh [1922].

Results obtained with sodium nitrite.

Figs. 6 and 7 show characteristic results in experiments in which nitrite was injected at low and high temperatures.

The following results were obtained from 14 such experiments, 6 at 10° C. and 8 at 20° C. (approx.).

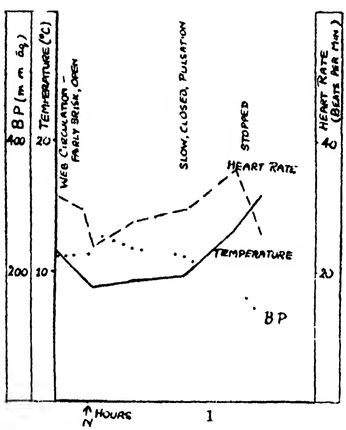

Fig. 6.

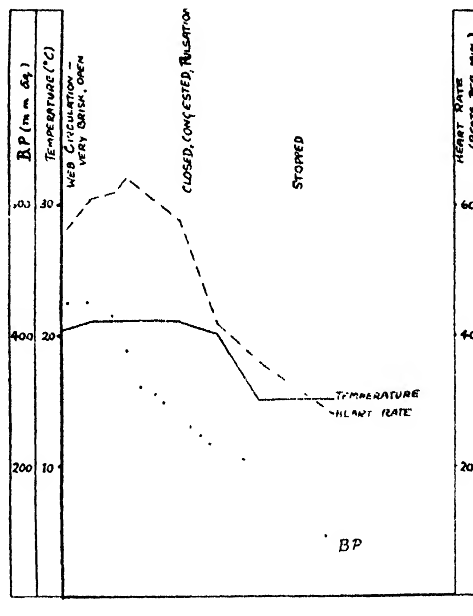

Fig. 7.

Fig. 6 Effects, at approx. 10° C., of injection, at N, of sodium nitrite 0·5 c.c., 2 p.c., on blood-pressure, heart rate and web circulation; showing breakdown of the heart rate and temperature relationship with rise of temperature.

Fig. 7. Effects, at 21° C., of injection, at N, of sodium nitrite 0·5 c.c., 2 p.c., on blood-pressure, heart rate and web circulation; showing failure to arrest these effects by cooling.

The **blood-pressure** was lowered in every case by nitrite, but the fall was relatively slighter and slower at low temperature. The pressure fell on an average by 30 p.c. in 15 min. at 20° C.; and by 10 p.c. in 15 min., by 29 p.c. in 1 hour, at 10° C.

The **heart rate** at 20° C. was slowed by nitrite in 7 cases (in 3 of which there was a preliminary slight rise), and slightly accelerated in 1 case. At 10° C. it accelerated in 4 cases and slowed in 2. In 4 cases at 20° C.

and 3 at 10° C. there was a late exaggerated fall in heart rate, associated with failure of the heart. In these cases the heart frequently became dissociated before arrest in diastole.

Upon the web circulation typical effects were produced by nitrite, slow flow, closed field, congestion and marked pulsation in active vessels leading to an oscillating movement of the corpuscles and ultimate arrest of circulation. These changes were produced, to a varying degree, in every

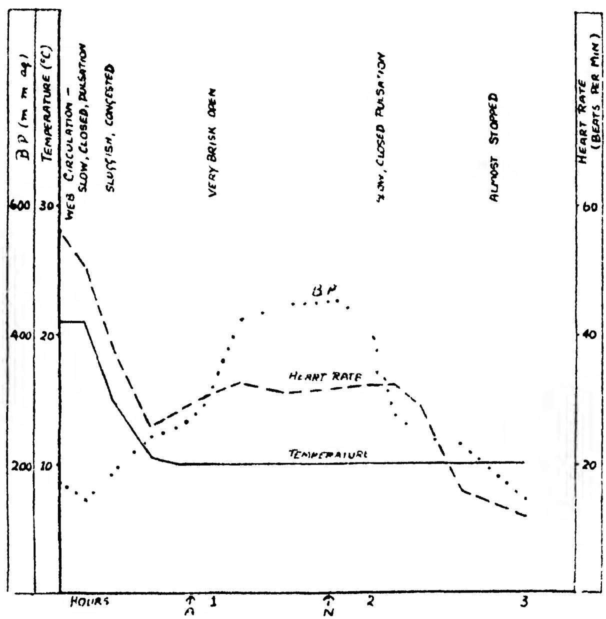

Fig. 8. Effect upon a developing nitrite action (injected 1 hour before the commencement of tracing) of cooling and of injection, at *A*, of adrenaline; and the subsequent effect of repeated nitrite, at *N*.

case. In some cases increase in the calibre of the vessels was observed, and a condition described as "vessels well delineated" was frequently noted. At 20° C. the arrest of circulation occurred in average time of 40 min.; at 10° C., 1 hour 15 min. At low temperatures the web circulation already tended, in some cases, to be slow, closed and congested, with visible pulsation. The effect of nitrite was thus masked by the "cold effect" and changes in web circulation appeared slighter at low than at high temperatures. The "cold + nitrite effect," however, unlike the "cold effect," was an irreversible change, which ultimately produced arrest of the circulation in every case.

Slight temporary improvement of the circulatory conditions was produced in 1 case out of 3 where high temperature was lowered, and in 1 case out of 3 where low temperature was raised, during a developing nitrite action. Injection of adrenaline during a developing nitrite action, at 20° C., produced in one case no effect; in a second a temporary improvement; and in a third case, when cooling accompanied the injection of adrenaline, a well-marked recovery (Fig. 8). Repetition of the nitrite injection caused subsequent rapid failure of the circulation in the two latter cases. Injection of adrenaline during a developing nitrite action at 10° C. produced in 4 cases a temporary improvement, but the typical nitrite effect supervened in each case.

Discussion of nitrite results.

The percentage fall in blood-pressure caused by nitrite is greater at high than at low temperature and the effect develops more quickly. This difference may be related to a difference in rate of absorption, or to a modification of the drug action itself. As in the case of adrenaline, the former explanation seems the more probable. In six experiments in which the frog's vessels were perfused with nitrite solution, at 10 and 20° C., the difference in temperature produced no detectable difference in the action of the drug.

The tendency of nitrite to slow the heart at 20°C., whereas it produces inconsistent effects at 10° C., may be related to the more rapid development of the drug effect at high temperature, and also to the fact that the heart is already slowed at low temperature. The average heart rate at onset, in this series, is 27 beats per min. at 10° C., and 54 at 20° C. The slowing appears to be associated with actual failure of the heart itself, although perfusion of nitrite solution, of comparable strength, directly through the heart, produces very little effect. With the circulation intact, a possible cause of injury to the heart may be the formation of methæmoglobin and other compounds by the action of nitrite on the hæmoglobin, as described by Haldane, Makgill and Mavrogordato [1896]. The blood from a heart arrested by nitrite gave a methæmoglobin spectrum.

The characteristic effect produced by nitrite on the web circulation has been already described. Direct application of nitrite solution to the web produces similar congestion and pulsation in the larger vessels. The change in the web circulation is more marked, however, when the nitrite is in the circulation, as the effects secondary to heart failure and fall of

blood-pressure are added to the direct effect of nitrite on the web vessels. At both high and low temperature nitrite, under these conditions, causes an injurious effect upon the circulation, which it is very difficult to modify; and, though the onset of the fatal termination is more rapid at high temperature, it is ultimately just as inevitable at low temperature.

SUMMARY.

1. Preliminary experiments upon the influence of temperature on the blood-pressure, heart rate and web circulation of the frog, when working with the intact circulation, are reported.

2. An experimental method of maintaining these factors constant, over periods suitable for the investigation of drug effects, is described.

3. The characteristic effects of adrenaline and of sodium nitrite upon the web circulation, at high and low temperatures, are described, and their relationship to the other circulatory factors is discussed.

4. It is suggested that the differences in these effects at high and low temperatures are due to a difference in the rate of absorption of the drug.

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612.621:612.018

NOTE ON THE EFFECT OF AGE ON THE RESPONSE OF IMMATURE MICE TO URINE OF PREGNANCY.

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I. Introduction.

Smith and Engle [1927] pointed out that the number of implantations of anterior pituitary substance necessary to induce sexual maturity in immature mice is inversely proportional to their age. Thus three daily implantations were required to produce maturity in 17-day-old mice, while five daily implantations were required in 15-day-old mice.

In testing gonad-stimulating hormones the only criterion generally observed is that the animal used is immature; there is no agreement amongst workers as to the age or weight of the immature animal employed. Thus Zondek and Aschheim [1927] used 6–8 g. mice corresponding to an age of 3–4 weeks. Engle [1929] used mice 18–20 days old, Wiesner and Marshall [1931] mice 3–4 weeks old with weights which varied between 8·5 and 15 g., and Evans and Simpson [1929] mice 24–25 days old. The fact that these and other workers have all used mice of varying age and weight may possibly explain some, at least, of the differences in the effects produced in the immature ovary by anterior pituitary hormones.

The present work was undertaken in order to determine more precisely the effect of the age of the animal on the sequence of events produced in the ovary by the gonad-stimulating hormones, especially with reference to normal ovulation and the formation of corpora lutea vera.

II. Technique.

Injections. For these experiments two series of immature mice were used; one series (LAP) being 21 days old, and the other (EAP) 14 days old at the first injection. The mice were injected subcutaneously with

0·1 c.c. of urine of pregnancy for a varying number of days and killed on the day following the last injection.

The urine of pregnancy used for the injections was collected from University College Hospital each day and all the experiments were done at the same time, except for two groups of mice in the EAP series, receiving six and seven injections respectively, which were injected at a later date. Owing to the difficulty of preserving the urine in good condition for any length of time, a fresh sample of urine was used every day, and it is thus unlikely that the results in these two groups show any discrepancy due to being done at a different time.

Histological methods. The ovaries were fixed in Bouin's fluid as soon as they were removed from the animal, serially sectioned at 7μ, and stained with Meyer's hæm-alum and eosin.

As an expression of the size of the maturing follicles and corpora lutea present in the ovaries, the maximum dimensions of each structure were measured in two directions at right angles, and the average was taken as the mean diameter. In order to have some means of comparing different animals, it was necessary to arrive at a value in terms of maturing follicles and corpora lutea for each pair of ovaries. For this purpose the average size of the largest normal follicles above resting size and of the largest corpora lutea, up to four in number, was determined for each animal. Where there were less than four normal follicles or corpora lutea, the number from which the average was taken is noted in brackets in Table I.

III. Effect of age on ovarian response.

The results of the experiments are summarized in Table I. Col. 1 shows the number of injections. It will be seen that for each period of injection several animals were killed. The measurements of the maturing follicles and corpora lutea are given in cols. 3, 5, 8 and 10. The averages of these figures for each group of animals relating to one period of injection are shown in cols. 4, 6, 9 and 11 and are plotted against the number of injections in Fig. 1.

Growth of the follicles and ovulation. In Fig. 1 the curves *a* and *b* show the growth of the follicles in the 21-day-old mouse and in the 14-day-old mouse respectively. In the 21-day-old mouse the growth of the follicles is rapid and reaches its maximum after four injections. In the 14-day-old mouse the growth is slower, as shown by the more gradual slope of curve *b*. In the younger animals the follicles, even when apparently mature, do not attain the size of those of the older series of mice. Ovulation takes

TABLE I.

(1) No. of daily injections	(2) EAP No. Injections begun at 14 days	(3) Average diameter of follicles for each animal (μ)	(4) Average diameter of follicles for group (μ)	(5) Average diameter of corpora lutea for each animal (μ)	(6) Average diameter of corpora lutea for group (μ)
2	—	—	—	—	—
3	9	297 (1)		None	
	10	None	297	,,	—
	11	,,		,,	
4	6	285 (3)		,,	
	7	355	327	,,	—
	8	342 (1)		,,	
5	4	391		,,	
	5	393 (3)	392	,,	—
6	93	405 (1)		306 (1)	
	94	393 (3)	398	450 (1)	414
	95	396 (1)		486 (1)	
7	96	429 (3)		441 (2)	
	97	477	437	405 (1)	414
	98	405 (1)		396	

(1) No. of daily injections	(7) LAP No. Injections begun at 21 days	(8) Average diameter of follicles for each animal (μ)	(9) Average diameter of follicles for group (μ)	(10) Average diameter of corpora lutea for each animal (μ)	(11) Average diameter of corpora lutea for group (μ)
2	7	267	267	None	—
3	3	333 (2)		,,	
	5	360 (1)	423	,,	—
	4	522 (1)		,,	
	6	479		,,	
4	1	495	495	403	403
	2	None		None	
5	8	506		533	
	9	459 (2)		508	
	10	495 (2)	457	535	514
	11	355 (2)		522	
	12	471 (3)		475	
6	14	342 (2)		481	
	15	364 (2)		542	
	16	391 (2)	365	502	505
	17	1 cystic		463	
	18	,,		537	
7	—	—	—	—	—

place in the 21-day-old mouse after four injections, coincident with the maximum size of the mature follicle. In the 14-day-old mouse ovulation first occurs after six injections, even though at this time the follicles still appear to be growing and have not yet reached the size necessary for ovulation in the normal animal.

Size of corpora lutea. The corpora lutea formed after ovulation in the

21-day-old mouse appear perfectly normal, and their average size when first observed (400μ) compares well with that found by Deanesly [1930] for the young corpora lutea of the normal mouse. The development of the corpora lutea in the 21-day-old series is shown by curve *c* on the graph. As the corpora lutea develop there is a decided falling off in the size of the

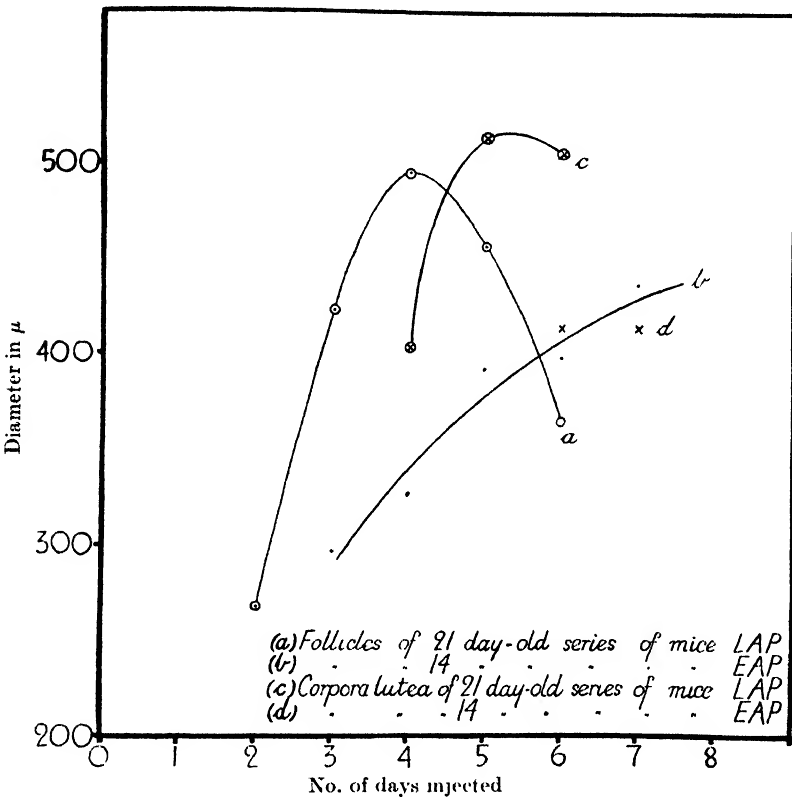

Fig. 1. Appearance and growth of the Graafian follicles and corpora lutea in the immature mouse following injection of urine of pregnancy.

growing follicles. In the 14-day old mouse the corpora lutea also appear normal and average about 400μ in size at their first appearance. However, it seems unlikely that the corpora lutea produced with further injections in this series would equal the maximum size of those of the 21-day-old series of mice.

Differences in the total effect. The age of the animal influences not only the maximum size of the follicles and corpora lutea but also the number of these structures actually developed. Thus, the total effect of the in-

jections appears to be greater in the mice of the 21-day-old series (LAP) than in those of the 14-day-old series (EAP). Though ovulation does occur in both the LAP and EAP series of mice after four and six injections respectively, it occurs more freely in the LAP series. The ovaries from this older series of mice contain therefore many normal corpora lutea, with few maturing follicles, whereas the ovaries from the younger series contain a number of maturing follicles and comparatively few normal corpora lutea, the luteinization here taking the form of corpora lutea atretica rather than of normal corpora lutea. The corpora lutea atretica in the 14-day-old series of mice first appear after six injections. They are also to be seen in the 21-day-old series after five injections but are not very numerous. In general, the difference between the two series of ovaries is similar to that found between groups of mice of the same age receiving different doses of urine. It is evident, therefore, that accurate standardization of the age is essential in the use of the immature animal for the assay of the ovary-stimulating substances, especially when a separation is being attempted. For this purpose, the older animal (21 days) appears to be the more satisfactory test animal, in that it gives a better defined result than the younger animal.

IV. Summary.

Twenty-one-day-old mice ovulate after four daily injections of 0·1 c.c. urine of pregnancy (human), whereas 14-day-old mice require six injections. Careful standardization of the age is therefore necessary in the use of such animals for the assay of the ovary-stimulating substances.

I wish to express my thanks to Dr A. S. Parkes for help and advice during the course of this work.

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FURTHER OBSERVATIONS ON THE EFFECTS OF SOME COMPONENT OF CRUDE LECITHINE ON DEPANCREATIZED ANIMALS.

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In two previous communications from this laboratory [Hershey, 1930; Hershey and Soskin, 1931] the remarkable effects of crude egg yolk lecithine upon the symptoms and signs exhibited by depancreatized dogs under certain conditions have been discussed. Since the depancreatized animals have been kept from 3 to 11 months before the typical condition which we believe is largely attributable to failure of liver function developed, the progress of the research has necessarily been very slow. During the past three years, however, we have had the opportunity of confirming and extending the experimental results previously reported.

METHODS.

The general procedures used in studying the diabetic animals have been the same as those discussed in previous communications. These animals need a great deal of care and attention, and the results of the chemical determinations on samples of blood or urine would be of little value if they were not considered with reference to the history and general condition of each animal at the time the determinations were made. To save space, a detailed report of these matters will not be included here. After the complete removal of the pancreas each animal was placed upon a diet of 300 g. of lean beef muscle and 100 g. of sucrose daily. This was the diet received by all the animals mentioned in Table I during the experimental periods which will be under discussion. The entire ration was in all cases completely consumed. The dose of insulin, which was administered subcutaneously twice a day, was adjusted in each animal after pancreatectomy to permit a slight or moderate glucosuria. The insulin dosage was usually constant throughout each experiment, and the ex-

ceptions to this noted below are considered separately. Blood sugar, urinary sugar and urinary nitrogen were determined by the Shaffer-Hartmann, Bertrand and Kjeldahl methods respectively.

Experimental results.

The actual figures which it is necessary to report are presented in Table I. The results which these figures help to illustrate may be discussed under several headings.

Table I.

Dog No.	Dates	Materials added to lean meat and sugar diet	Daily average of sugar excretion (g.)	Daily average of nitrogen excretion (g.)
10	(Depancreatized October, 1929)			
	1–4. xi. 29		4·5	—
	9. xi.–2. xii. 29	Lecithine	15·2	—
	3–6. xii. 29		12·4	5·6
	7–19. xii. 29		5·5	5·3
	8–22. ii. 30	Lecithine	2·4	6·2
	Dog killed Feb. 25, Liver fat 2·5 p.c., iodine No. 125.			
11	(Depancreatized May, 1930)			
	20. i–12. ii. 31	Vitamins A, B, C, D	1·2 (High 1·9)	8·1
	17. ii–8. iii. 31	Lecithine	5·0 (High 13·4)	6·4
15	(Depancreatized March, 1929)			
	14–20. xii. 29		3·1	5·3
	31. xii. 29–4. i. 30		2·2	4·5
	7–20. i. 30	Lecithine	17·0	5·6
	11–21. ii. 30		4·3	5·6
	23–30. xi. 30	Suet	1·1	—
	30. i–3. ii. 31	Suet and lecithine	4·6	—
16	(Depancreatized February, 1930)			
	14. xi–1. xii. 30		14·5	—
	30. xii. 30–8. i. 31	Vitamins and suet	4·3	—
	16–24. i. 31	Lecithine and suet	10·5	—
	27–31. i. 31	Suet	1·3	—
	7–21. ii. 31	Lecithine and suet	7·0	—
	1–25. iii. 31	Suet	1·0	—
17	(Depancreatized December, 1930)			
	31. i.–12. ii. 31		4·6	5·9
	13–23. ii. 31	Lecithine	9·2	—
	24. ii.–21. iii. 31	Lecithine and suet	15·4	6·4

The effects of lecithine on the general condition of the animals. The most interesting aspect of the effect of lecithine is probably the relief of a condition which in many instances borders upon a moribund state. It requires considerable experience to recognize this condition in its early stages, since the animals may only exhibit slight weakness and, in some

cases, jaundice. Liver function tests have not as yet been conducted. Later the weakness may be very marked, but it is not expedient to permit the development of this stage of the condition if it is desired to demonstrate the recovery of the animals when lecithine is provided. The falling off of the sugar excretion is the most valuable sign. Within a few days after lecithine is given the animals usually appear definitely stronger and brighter. These effects were observed in all the animals referred to in Table I. Several of the depancreatized animals which we have studied in this investigation have lived for very long periods after complete pancreatectomy. The longest record is that of dog No. 15, which lived for more than $2\frac{1}{2}$ years after the removal of its pancreas. On numerous occasions its condition was critical, but the addition of lecithine to the diet restored it apparently to normal health. The terminal illness was not associated with any signs of failure of liver function.

The effect on sugar excretion. It usually happens that the sugar excretion of the depancreatized animal after the first week or 10 days following the operation reaches a fairly constant level, which may be maintained with minor fluctuations for several months. During this time the animal is usually in very good health. When the characteristic condition which is associated with fatty infiltration and degeneration of the liver appears, the animals become weaker, bile pigments may appear in the urine, and the sugar excretion may be greatly diminished. It is sometimes necessary to decrease the dose of insulin to avoid fatal hypoglycæmia from a dose which previously permitted glucosuria. We have not included any figures in Table I for periods in which the insulin dosage had to be decreased. In all the animals referred to in this table there is convincing evidence that some component of the crude egg yolk lecithine added to their diet produces a very definite increase in the sugar excretion. This increase in sugar excretion is not accompanied by any very definite change in urinary nitrogen. Although we do not intend to discuss at length the bearing of these results on the vexed question of sugar production from fat, it is very difficult to account for the production of the great excess of sugar excreted in some cases during the period of lecithine feeding by any other mechanism. For example, in the experiment on dog No. 15, the average daily sugar excretion before the administration of lecithine was not over 3 g., while during the lecithine period of 13 days the average excretion was 17 g. It is very difficult to believe that the carbohydrate reserves have provided this excess sugar. From experiments on other depancreatized animals we have reason to believe that the liver and muscle glycogen are quite as high or higher during the

lecithine period as during the time when the sugar excretion is lower and the animals are suffering from other symptoms which are probably also attributable to disturbed liver function. The figures for nitrogen excretion indicate that little or none of this excess sugar is derived from protein. The question arises as to whether or not 10 g. of lecithine, which was the amount administered each day to these animals, could be the source from which the extra sugar is formed. If lecithine could be converted into dextrose in the animal body it is obvious that a large part of the excess sugar might be provided by the amount of phospholipine available. While further studies, in which a different plan of attack will be necessary, are required to answer these questions, the findings mentioned in the next section may furnish a clue. Unless the state of the carbohydrate reserves of the individual animal is accurately known it is obvious that experiments of the type reported here cannot provide unassailable evidence for sugar production from fat.

Failure of lecithine to increase sugar excretion. On only two occasions during the last three years have we encountered conditions in which lecithine failed to increase the sugar excretion of animals which were in a condition that previous experience led us to expect would be suitable for this demonstration. One case is referred to in the experiment on dog No. 10. The first time lecithine was administered to this animal a very definite increase in sugar excretion resulted. Later, however (February 8–22). 10 g. of lecithine daily failed to raise the sugar excretion. The animal was in good condition, and was sacrificed on February 25. The liver contained 2·5 p.c. fatty acid, which had an iodine number of 125. These values, and the general appearance of the liver, are typical of the lecithine livers. It is possible that the sugar excretion was not increased because there had been insufficient opportunity, previous to the second administration of lecithine, for the production of those changes which are responsible for the accumulation of fat in the liver.

The effects of vitamins. Fairly satisfactory evidence has been obtained in this series of experiments that the effect of lecithine is not due to the presence of vitamins. Some experiments, indicating that vitamins A and C are not present in the preparations of lecithine we have used, have been reported previously. In the experiments on dog No. 11 adequate amounts of vitamins A, B, C and D in concentrated form were provided during a period when the animal continued to excrete very little sugar. A definite rise in sugar excretion occurred subsequently, however, when lecithine was provided. The vitamins were also administered to dog No. 16 without any trace of the lecithine effect being observed.

Acceleration of the onset of the characteristic condition by fat feeding. In the experimental results secured from the observations made on dogs 15, 16 and 17, convincing evidence is provided that the onset of the condition characterized by lowered sugar excretion can be accelerated by the feeding of fat. Beef suet, approximately 10 g. daily, was usually effective within a few weeks in producing the desired condition. Great care must be exercised, however, in feeding this material, as some dogs are unable to tolerate more than very small amounts at first. Some refuse to eat sufficient fat to produce the required effect. The results of one experiment also suggest that when beef suet and lecithine are administered together, the sugar excretion is increased to a greater extent than when lecithine alone is given (dog No. 17). Further work is, of course, necessary to establish the significance of this result. The amounts of lecithine and suet provided are of course kept constant throughout any one experiment. These experimental results do furnish evidence, however, that a condition which appears to be very similar to that observed after prolonged periods in depancreatized animals kept on a sugar and lean meat diet, can be produced much more quickly by the addition of fairly saturated fats to the diet.

The effect of fat feeding in diabetic animals. The very definite decrease in sugar excretion produced by adding large amounts of saturated fats to the diet is well illustrated in the experiments on dogs 15 and 16 (Table I). In certain experiments where fat was fed the fasting blood sugar level became so low and the sugar excretion diminished to such an extent that the dose of insulin had to be decreased. None of these results is included in the table. The diet of certain of these animals was changed slightly and we were able to show that fat feeding may affect a completely depancreatized animal in such a way that it is able to tolerate a diet of fairly high caloric value without exhibiting glucosuria or hyperglycæmia when no insulin is administered for fairly long periods. One result of this kind is summarized in the following protocol.

Dog No. 11, weight 4·0 kg. This depancreatized animal had been excreting varying amounts of sugar on a daily diet of 300 g. of lean meat and 80 g. of sucrose (insulin 10 units). The excretion was approximately 1 g. per day. When fat was added to the diet the sugar output was definitely less and dangerous levels of hypoglycæmia were sometimes observed. On June 4, the diet was changed to the following: 300 g. lean meat, 20 g. beef dripping (iodine number about 40). No insulin was given. For the next 3 weeks the sugar excreted was less than ½ g. a day. On many days only a trace of sugar was present. From June 25 to July 17, lecithine was added to the lean meat and fat diet, but in this instance (the second that we have observed) no increased sugar excretion was produced. That is, for 6 weeks the animal received no insulin. Fasting blood sugars determined on several occasions during

the 6 weeks showed values within the range found in normal dogs. During all but the last few days of this 6 weeks' period, when the animal suddenly became weak, it was apparently in excellent condition. No trace of pancreas was found at autopsy.

We suggest that the explanation of these results is that the fat feeding produces changes in the liver which inhibit gluconeogenesis in this organ to such an extent that the blood sugar is only slightly raised above the normal level, and very little sugar appears in the urine. These animals are apt to die very suddenly, however, and the livers are then found to be very friable and to contain large amounts of saturated fatty acids. A possible application of these results to treatment of diabetics in clinics where large amounts of saturated fats are permitted is obvious. It is possible to improve the "chemistry" of the diabetic organism without improving the clinical condition. It is remarkable, however, that depancreatized dogs without insulin may appear perfectly bright and normal for a month or longer, or without hyperglycæmia and glucosuria before they succumb to the effects of the liver damage. Since a series of depancreatized animals are at present under observation in an attempt to gain more information on these points, further discussion of this aspect of the subject can be reserved for a subsequent communication.

Histological studies. Specimens of the livers from animals which developed the characteristic condition without fat feeding, or livers from animals which have been fed large amounts of saturated fat, may show fatty degeneration as well as infiltration. The livers of the animals which had received lecithine are free from detectable fatty degeneration. A detailed account of these changes and those encountered in the normal animals referred to in the following papers will be given when the histological work is completed.

Summary.

The alleviation of a critical condition, characterized by signs of failure of liver function, by the addition of lecithine to the diet of depancreatized animals has been repeatedly demonstrated. These results confirm previous observations from this laboratory. Very satisfactory evidence has been obtained that the sugar excretion of those animals which developed the characteristic condition is greatly increased by the administration of lecithine. The source of this extra sugar has been briefly discussed. The action of the lecithine is not due to the presence of vitamins A, B, C or D. A condition similar to that which occurs spontaneously in animals kept on a lean meat and sugar diet can be produced

in a relatively short time by the addition of fairly saturated fats to the diet. The results of the studies on normal animals bear so directly on the questions raised by this one that a detailed discussion of the action of lecithine in diabetic animals will be reserved until certain experiments suggested by the recent findings have been completed.

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THE EFFECT OF LECITHINE ON FAT DEPOSITION IN THE LIVER OF THE NORMAL RAT.

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THE very interesting effects of some component of crude lecithine upon the condition of diabetic animals have been discussed in previous communications from this laboratory [Hershey, 1930; Hershey and Soskin, 1931; Best and Hershey, 1932]. The symptoms exhibited by these animals and the autopsy findings indicate that the characteristic condition is largely attributable to failure of liver function. The results of these studies suggested that an investigation of the effect of lecithine on deposition of fat in the livers of normal animals might be profitable. The literature relevant to this subject has been reviewed in the monographs by Leathes and Raper [1925] and by MacLean and MacLean [1927]. As far as we know, the particular problem in which we are interested has not been investigated by previous workers. The experiments were planned with a view to obtaining deposition of large amounts of fat in the livers of a group of control animals. This was accomplished by feeding a diet high in fairly saturated fats. A second group of animals comparable in every way, as far as we could determine, with the controls, received the same amount of fat as those in the control group and *in addition* varying amounts of crude or purified lecithine. The results demonstrate, among other points, that crude and purified lecithine influence the accumulation of fat in the livers of the test group of animals.

METHODS.

White rats of the Wistar strain, weighing between 150 and 230 g., were used as test animals. Previous to the experiment they had been receiving a stock diet which was adequate in all respects. All the animals used in the experiment were apparently healthy, and great care was taken to ensure that the various groups into which they were divided for any one experiment were as similar as possible. Much time was wasted

in preliminary experiments in which attempts were made to use groups of animals in one cage, but this procedure was not found to be feasible. In all the experiments reported in this paper individual cages were used, and notes on the condition of each animal were made throughout the experiment. Every animal in any experiment ate approximately the same amount of the stock diet, which has the following composition: 32·5 p.c. of each of the following—whole cracked wheat, rolled oats and corn meal, and 2·5 p.c. of bone meal. The amounts of fat and of the lecithine under test are given in each of the tables or summaries. In all cases the fat was provided in the form of beef dripping, which has an iodine number of approximately 40. The food was prepared daily. The dripping was melted and added to the stock diet. The mixture was then heated in a boiling water bath for 2 hours, with intermittent stirring. The stirring was continued during the subsequent cooling to ensure the equal distribution of the added fat. The lecithine was thoroughly mixed with the other food by prolonged grinding. The diet for each rat was carefully weighed each day, and in cases where food was left in the cage at the end of 24 hours the amount was determined by weighing. The food trays were designed so that there was little or no food spilled, and the construction of the cages made it possible to recover any spilled food without difficulty. Fat estimations on the food residue showed that the fat had been thoroughly mixed with the other constituents. At the end of the experiment the animals were stunned and decapitated. The animals were not fed on the day on which they were killed. Blood samples were collected from the severed carotid arteries. The livers were removed as soon as the animals were dead, and the content of fatty acid determined by the Leathes and Raper modification of Liebermann's saponification method. The iodine number of the fatty acid of the liver was determined by Wijs' procedure, as outlined by Leathes and Raper. The phospholipines of the liver and blood were calculated from the phosphate content as determined on the alcohol-ether soluble portion by the Briggs' modification [1922] of the Bell-Doisy method. Glycogen was estimated by the Pflüger technique.

Experimental results.

Egg yolk lecithine. In the first experiment ten rats were used in each of the two groups. The details of the experiments and the results are shown in Tables I and II. The egg yolk lecithine used in these experiments was secured from a commercial firm and was prepared from dried egg yolk, after removal of the fats with benzene, by alcoholic extraction of

TABLE I. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Wt. of liver (g.)	Fatty acids in liver (p.c.)
1	36	99	2·8	170	188	7·77	20·50
2	36	95	2·6	154	167	5·02	5·88
3	35	91	2·6	152	160	4·71	4·20
4	36	94	2·6	178	175	5·58	4·05
5	35	97	2·8	155	167	4·42	5·52
6	36	94	2·6	135	144	4·78	5·12
7	38	100	2·6	121	135	4·51	3·67
8	38	100	2·6	150	144	5·22	6·87
9	38	100	2·6	149	152	6·44	11·30
							Average = 7·36

TABLE II. Fat diet with crude egg lecithine. Stock diet with fat (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Wt. of liver (g.)	Fatty acids in liver (p.c.)
11	35	102	2·9	181	185	1·4	7·32	3·20
12	36	89	2·5	149	150	1·2	4·57	3·78
13	36	90	2·5	162	157	1·2	6·33	3·38
14	38	103	2·7	193	189	1·3	7·34	3·09
15	38	93	2·4	154	148	1·2	6·86	3·29
16	35	96	2·7	190	182	1·3	7·61	3·30
17	35	91	2·6	144	135	1·3	6·37	3·75
18	38	96	2·5	140	147	1·2	5·48	3·12
19	38	99	2·6	176	170	1·3	6·59	3·97
20	38	108	3·1	185	192	1·5	6·85	3·50
								Average = 3·38

the phospholipines and subsequent removal of the alcohol *in vacuo*. The results of the experiments in which this material was used appear to demonstrate in a convincing manner that some component of lecithine modifies the deposition of fat in the livers of the test animals. The figures also illustrate the very important fact that there is a tremendous variation in the deposition of fat in the livers of different members of a group of animals, each of which is receiving approximately the same amount of fat in the diet.

In most of the experiments all the rats consumed a satisfactory amount of fat. In certain cases, however, the quantity eaten by some of the test animals was not of the same order as that ingested by the controls. The figures for these animals have not been included in the results.

The effects of smaller amounts of lecithine. To study the effects on fatty acids of the liver when small amounts of lecithine were provided, a group of thirty white rats was used. The animals were divided into three groups of ten each, care being taken that the groups were as similar as possible.

Ten rats received 6·15 g. of stock diet *plus* fat 3·5 g. (beef dripping, iodine number 40). Fat formed 40 p.c. of the total food. The experiments lasted 26 days. The average amount of fat taken daily was 3·4 g. The average weight of the rats at the beginning of the experiment was 190 g., and at the end 182 g. The percentage of fatty acids in the livers varied from 7·0 to 25·3 p.c. The average was *15·7* p.c. The iodine numbers varied from 78 to 108. The average was 95. One extraordinarily high iodine number was probably due to an error, and that result is not included in this average.

In the second group all rats received the same amount of stock diet and fat, and in addition egg yolk lecithine. The lecithine formed 9·4 p.c. of the total food. The average figure for the fat eaten daily was 2·97 g., while that for the lecithine was 0·85 g. The average weight of the rats at the beginning of the experiment was 191 g., and the average weight at the end was 177 g. The percentage of fatty acids in the liver varied from 3·3 to 5·1. The average was *3·7* p.c. The iodine number varied from 101 to 136, the average was 129.

The diet for the third group was exactly the same as that of the second, except that lecithine formed 4·9 p.c. of the diet instead of 9·4 p.c. The average figure for the amount of food eaten daily was 3 12 g., while the average figure for the lecithine was 0·44 g. The average figure for the weight at the beginning of the experiment was 191 g., and at the end 187 g. The percentage of fatty acid in the liver varied from 3 1 to 8·2 p.c. The average figure was *4·6* p.c. The iodine numbers varied from 83 to 107. The average figure was 98.

These results appear to furnish evidence that as little as 0·44 g. of crude egg yolk lecithine daily is sufficient to prevent the deposition of large amounts of fat in the liver of a rat under the conditions of these experiments. It will be observed that the rats in the test groups in this series did not eat quite as much added fat as the controls. We have no hesitation in reporting these results, however, for the following reasons: (1) In many other experiments rats from the same colony have invariably shown as high an average fat content of the liver as the test animals in this particular experiment. In these other experiments the controls have eaten even less fat than that taken by the test animals of this experiment. (2) The figures for the individual rats show that many of the animals did eat as much fat as the controls. (3) The test animals ate a certain amount of fatty material in the crude lecithine, which can perhaps be placed to their credit. (4) Furthermore, a study of the tables which contain the results of the determinations made on the control rats demonstrates the fact that the extent of the deposition of liver fat is not proportional to the amount of fat eaten when this amount varies between 2 and 3·5 g. per day. For example, in Table IX the rats which ate 2·3 g. of fat daily showed a much higher fat content than several which ate 2·5 g.

The effects of purified lecithine. Crude egg yolk lecithine was purified by the cadmium chloride procedure of Levene and Rolf [1927]. This fractionation was carried out by one of us (M. E. H.) in the Department of Biochemistry under the direction of Prof. H. D. Kay. The purified lecithine (iodine number 65) obtained was tested on a group of animals.

The results are shown in Tables III and IV. These results establish the fact that purified egg yolk lecithine exerts the same effect as that produced by the crude material.

TABLE III. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	22	54 55	2·48	300	253	6 35	105
2	22	54·55	2·48	202	174	19·2	100
3	22	54·35	2·47	190	194	27·6	—
4	22	54·20	2·46	179	171	19·3	97
5	22	53·60	2·43	189	172	16 2	102
6	20	48 00	2·40	180	177	20·5	95
7	22	54·60	2·48	177	180	15·9	96
8	22	52·90	2·40	169	166	28·2	102
9	22	54·10	2·45	162	160	12·5	104
10	22	53·80	2·44	150	150	17 2	77
						Average = 18·3	

TABLE IV. Fat diet with purified lecithine. Stock diet with fat (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
11	22	52·80	2·40	278	257	0·48	3·49	137
12	22	53·60	2·43	203	177	0·49	3·65	107
13	22	49·50	2·35	190	150	0·47	7·4	113
14	22	52·30	2·38	180	171	0·48	3·15	117
15	22	53·00	2·41	199	186	0·48	3·98	118
16	22	46·90	2·13	184	164	0·43	3·58	124
17	22	48·70	2·22	180	166	0·44	3·74	139
18	22	52·80	2·40	170	167	0·48	8·4	76
19	22	51·60	2·34	160	155	0·47	4 05	129
20	22	53·90	2·44	160	151	0·49	4·11	128
							Average = 4·5	

Lecithine from beef liver. In the next series of experiments lecithine, prepared from fresh beef liver, was tested. The lecithine fraction was prepared in the following manner: fresh beef liver was minced and dried in a vacuum oven at low temperature. The dried material was ground and then thoroughly extracted with absolute ethyl alcohol. The material was filtered through paper and the filtrate evaporated to small volume in an efficient vacuum still. The residue was evaporated to a syrup in a large glass flask, and ether was then added until no more material would go into solution. The mixture was allowed to stand overnight in a cold room, and then filtered through paper. The clear ethereal solution was added with stirring to three volumes of acetone. After 48 hours the supernatant fluid was decanted, and the precipitate removed. This material was then

stored over paraffin in an atmosphere of nitrogen. This was the material which was subsequently purified by the cadmium chloride procedure. We are indebted to the Connaught Laboratories for the fresh beef liver, for the various reagents, and for the use of the large apparatus required in the preparation of the lecithine.

The effect of crude beef liver lecithine. The results (Tables V and VI) demonstrate that 1 g. and 0·5 g. of lecithine from this source daily are capable of preventing the deposition of fat in the liver of normal white rats.

TABLE V. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	21	52·5	2 5	189	181	29·6	110
2	21	52·5	2·5	171	173	14·5	109
3	21	52·5	2·5	170	172	20·3	116
4	21	52 5	2·5	226	206	25·1	102
5	21	52·5	2·5	164	165	9·2	110
6	22	54·0	2·45	175	167	21·2	100
7	22	55·0	2·5	173	169	16·6	115
8	22	55·0	2 5	160	160	7·8	133
9	22	55·0	2·5	158	154	20 7	63
10	22	55 0	2 5	150	142	7·9	138
					Average	=17·3	

TABLE VI. Fat diet with crude liver lecithine. Stock diet with fat added (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
32	22	52·4	2·38	209	200	0·95	3·1	120
33	22	54·2	2 47	160	168	0·99	4·6	116
34	22	53·7	2·44	172	188	0·97	4·9	175
35	22	51·8	2·36	192	187	0·94	9·5	172
36	22	51·2	2 33	150	143	0 93	4 4	246
37	22	54·2	2·47	151	123	0 99	4·1	192
38	22	51·6	2·35	202	193	0 94	5·1	194
40	22	53·5	2·43	162	175	0·97	3·6	215
41	22	53·2	2·42	170	172	0·48	4·1	205
42	22	55 0	2 50	181	190	0·50	4·0	212
43	22	53·7	2·44	186	192	0·49	5·7	168
44	22	52·5	2·38	165	167	0·48	3·7	213
45	22	53·2	2·42	181	175	0·48	5·7	175

Average 12·4 p.c. lecithine =4·7
6·7 p.c. lecithine =4·8

Purified beef liver lecithine. Some 340 g. of purified lecithine (iodine number 75) were obtained by fractionation of the lecithine according to the Levene and Rolf cadmium chloride procedure. Varying amounts of this lecithine were added to the stock diet in the manner described

above. The smallest effective daily dose studied was 0·1 g. The results of the experiment in which 0·25 g. was given daily are slightly more consistent, however, and these are given in Tables VII and VIII.

TABLE VII. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	20	50·0	2·50	0·17	210	200	9·2	97
2	20	50 0	2·50	0 15	190	184	11·0	95
3	20	50·0	2 50	0 16	170	174	16·0	90
4	20	50·0	2·50	0 15	170	166	8 5	94
5	21	52·5	2·50	0·17	180	176	21 1	—
6	21	52·5	2 50	0·16	176	174	8·2	100
7	21	52·5	2 50	0·16	180	170	11 7	95
8	21	52 5	2 50	0·17	182	170	28 2	97
9	21	51·5	2·46	0·18	166	152	13 8	89
10	21	52 5	2 50	0·14	162	158	16 2	91
							Average = 14·4	

TABLE VIII. Fat diet with purified liver lecithine. Stock diet with fat added (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
11	21	52·5	2·50	0 17	160	145	0·25	5·30	107
12	21	52·5	2 50	0·20	160	152	0 25	6 55	111
13	21	52·5	2·50	0 18	180	170	0 25	5 85	112
14	21	51·5	2·45	0 19	230	220	0 24	3 87	117
15	21	51·5	2 45	0 18	160	160	0·24	5 85	115
16	21	52·5	2 50	0·15	180	172	0·25	5·40	109
17	21	52 5	2·50	0·15	164	152	0 25	5·08	110
18	21	52·5	2·50	0 15	198	190	0·25	4 27	118
19	21	51·5	2 45	0 16	188	180	0 24	6·12	110
								Average = 5 76	

Proof that the excretion of fatty acids plays no part in the effects of lecithine. Although these results may be shown subsequently to have little physiological significance, they are of considerable interest provided that the effect of lecithine is not due to some relatively unimportant mechanism. The only obvious pitfall seems to be that the lecithine might increase the excretion of fat. In preliminary experiments in which the food residue and fæces were examined for fat, no evidence could be obtained that more fat was lost in the animals on lecithine than in others. In an attempt to make this point perfectly clear, however, the fat in the fæces has been estimated by the saponification method, for each of the members of control and test groups of animals, in three experiments. The fæces were collected daily and were immediately placed in the alkali.

The figures in Tables VII and VIII show that there is no difference in the fat excretion of the control and test groups, and they provide very strong evidence that fat excretion is not a significant factor in the interpretation of these results. In the other experiments in which phospholipines prepared from beef liver and egg yolk lecithine prevented deposition of liver fat, the fat excretion of the animals which received the lecithine was of the same order as that of those on the control diet. The figures for the experiment in which egg yolk lecithine was used are given in Tables IX and X. These results, in addition to the data on fat excretion which they provide, furnish further evidence of the effect of phospholipines derived from egg yolk on deposition of liver fat.

TABLE IX. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Total fat excreted (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	21	52·5	2·50	2·46	0 12	171	150	8·9	110
2	21	51 0	2·43	2 19	0·11	155	145	21·5	86
3	21	52·1	2·48	2·74	0 14	158	148	14·8	106
4	21	52·1	2 48	2·67	0 13	200	195	17·3	100
5	21	52·5	2·50	2 49	0 12	174	170	12·3	89
6	21	51 0	2 43	2·69	0 13	165	150	27·7	84
7	21	52 5	2·50	2 38	0 12	163	160	25·0	97
							Average =	18 2	

TABLE X. Fat diet with crude egg lecithine. Stock diet with fat added (fat 40 p c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g)	Fat eaten daily (g.)	Total fat excreted (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c)	Iodine No.
11	21	51 5	2 43	2·11	0·10	153	150	0·49	3·75	– –
12	21	48 7	2 32	1·65	0 08	177	162	0·46	3·50	133
14	21	52 0	2 47	3·16	0·16	181	175	0 49	3·32	140
16	21	50·0	2 38	2 33	0·12	161	137	0·48	3·15	137
20	21	51 6	2 45	2 27	0 11	150	148	0 49	3 50	114
								Average =	3·44	

Lecithine in liver and blood. Determinations of alcohol-ether soluble phosphorus on samples of liver and blood from control and test animals were very kindly made for us by Dr Kay of the Department of Biochemistry. The average liver value, calculated as lecithine, in four control animals was 2·94 p.c. In four test animals each of which ate approximately 0·85 g. of egg yolk lecithine daily the value was 3·38 p.c., and in four test animals which ate 0·44 g. of the same material 3·20 p.c. We are not prepared to conclude without further experiments that the difference

between control and test animals is significant, but it is interesting to note that a very large proportion of the total fatty acid in the livers of the test animals must have been derived from the phospholipines. The livers of test animals which contained 3·38 p.c. lecithine showed an average value of 3·85 p.c. fatty acid, those with 3·20 p.c. lecithine 3·80 p.c. fatty acid. The livers of the controls contained 2·94 p.c. lecithine and 11·2 p.c. fatty acids.

The results for the phospholipines, calculated as lecithine, for the blood samples from control animals demonstrate that these values are definitely lower than those obtained from the animals given lecithine. While the animals were not fed on the day on which they were killed, and while we are certain that some of the animals did not eat anything for at least 5 hours previous to their death, we cannot conclude that absorption of lecithine from the intestine did not influence these values obtained for the blood.

Glycogen. Glycogen determinations on samples of the livers of control and test animals have been made for us by our colleague Dr E. T. Waters. Both the fatty livers of the control and the relatively fat-free specimens from the lecithine animals may contain normal quantities of glycogen. The results thus far obtained do not suggest that there is any significant difference in the amounts of liver glycogen in the two groups.

Fatty acid content of whole animal. In several experiments the total fatty acid content of control and test animals has been determined by the saponification procedure. The figures suggest that the animals which had lecithine do not contain as much fatty acid as the controls. The difference is not great, however, and further experiments are required to settle the point. The iodine numbers of the fatty acids obtained from the bodies (livers excluded) of the control and test animals were of the same order.

Discussion.

A review of the literature shows that there is very little information available concerning the effects of diets rich in fat on the deposition of fat in the livers of various species of animals. In the experiments which we carried out during the hot summer weather, difficulties were encountered in securing a high average fat content in the livers of white rats. During the autumn and winter, however, a daily ration containing approximately 2·5 g. of fairly saturated fat has consistently produced a high average fat content in the livers of the control animals (Tables I, III, V, VII and IX). These results are so consistent that it might be considered unnecessary to provide a group of control animals for every new

test if one could be absolutely certain that the rats were from the same colony and had received the same diet and attention. It is certain, however, that rats from one colony cannot be used as controls on those from another. In one experiment in which a series of rats from a new colony was used the results had to be discarded because we made the mistake of assuming that the control group would exhibit fatty livers on the same diet as that received by the control rats referred to in the above tables. None of the rats from the new colony which were used as controls had a high liver fat. The cause for this great difference between rats from various colonies would be an interesting subject for further study.

The results of the experiments reported in this paper provide evidence that the addition of crude and purified lecithine from egg yolk and from fresh beef liver to the diet of normal white rats prevents the accumulation of liver fat. These results are very clear-cut. The evidence appears satisfactory that the effect is not due to excretion of fat by the animals on the "lecithine" diet. The fæces were carefully examined by one of us each day, and it appeared probable, even before the actual estimation of the fæcal fat in the control and test groups had been made, that fat excretion by the test animals was not a significant factor in the interpretation of the results. It is in the highest degree unlikely that the saponification method would fail to detect fatty material in one case and not in the other. It is also most improbable that the fat in the intestine of the lecithine-fed animals would be decomposed beyond the fatty acid and glycerol stage.

In the determination of the iodine numbers of the fatty acids from various sources the reagents and the technique have been repeatedly checked by determination of the iodine absorption of a sample of Merck's oleic acid. The iodine number of the beef dripping has ranged between 39 and 41. The tables demonstrate that the iodine number of the fatty acids from the livers of the lecithine-fed animals are on the average considerably higher than those of the control groups. While the average figures may have no great significance in this connection, calculation of these values from the experiments which are reported in this paper shows the following result—control rats 100, lecithine-fed rats 132. The iodine number of the fæcal fat varied between 20 and 50, and was usually between 30 and 40. An interesting point arising out of the determination of the iodine numbers is the very high figures obtained for the fatty acids of the liver of certain of the rats which received lecithine prepared from beef liver. These estimations were made with the same reagents and at the same time as those on the control animals. These high

figures suggest that the livers of certain of the animals receiving crude lecithine may contain a large proportion of highly unsaturated fatty acid, but further study is required on this point. If this result can be confirmed however in experiments on larger animals, a good opportunity might be provided for the isolation and identification of the fatty acid or acids responsible.

In an investigation of the effects of the components of lecithine on deposition of liver fat the results of which will be published shortly by two of us (C.H.B. and M.E.H.), evidence has been obtained that the active component of lecithine is choline. In view of this finding a discussion of the physiological significance of the results given in this paper will not be included here.

Summary.

When white rats weighing between 150 and 230 g. receive a daily ration containing 2·5 g. of fairly saturated fat (iodine number approximately 40) for 3 weeks, their livers may be found to contain very large amounts of fatty acids. The average value of the iodine number of these fatty acids in our experiments was approximately 100. Comparable groups of animals receiving the same diet as above, and in addition varying amounts of crude or purified lecithine prepared from egg yolk or beef liver, do not exhibit this increase in liver fat. The values obtained may be as low or lower than those of animals fed on a normal mixed diet. The average value of the iodine numbers of the fatty acids of this group was approximately 132. No evidence could be obtained that increased excretion of fat plays any part in the interpretation of the effect of lecithine.

The results of a preliminary investigation of the effects of lecithine upon liver glycogen and phospholipines, and upon the distribution of fat in other parts of the bodies of the test animals, are briefly discussed.

We would like to express here our appreciation of the expert technical assistance given us by Miss G. I. Harpell.

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CHANGES IN THE BLOOD COMPOSITION OF UN-ANÆSTHETIZED RABBITS FOLLOWING THE INGESTION OF WATER AND SALINE[1].

With special reference to the distribution of fluid between plasma and corpuscles and to the relationship between blood composition and diuresis.

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Introduction.

For some time it has been a matter of dispute whether, following upon the administration of water by the alimentary tract, there is or is not a blood dilution. Thus Haldane and Priestley [1916], Priestley [1916, 1921], Engel and Scharl [1906] consider that no appreciable change in blood concentration takes place and in contrast Blix [1916], Marx and Mohr [1927], Bayliss and Fee [1930], Rioch [1930] detect considerable dilutions. Haldane and Priestley [1916] note a constancy of the hæmoglobin percentage in man, but in a subsequent paper Priestley [1916] describes a decrease in the electrical conductivity.

In these results there may be nothing essentially contradictory. The type and breed and diet of the animal used, the dosage of water, the substance used as an index of blood dilution, and the times after water administration at which the blood samples are taken may determine these differences. Further, the differences in physiological response induced by such procedures as the administration of anæsthetics and decortication or decerebration are of at least equal importance.

Dilution of the blood is usually estimated by observing the changes in percentage concentration of some substance which is selected as a measure of dilution. In this paper the percentage of solid substance and the hæmoglobin content of whole blood, the hæmatocrit reading, the protein content of plasma, and the chlorine content of plasma have been used for this purpose. Although it is not methodically difficult to detect even small changes in the concentration of these substances it is by no

[1] Communicated to the Medical Research Society on October 23, 1931.

means so easy to decide that such changes are the result of water absorption. Thus, Barcroft has shown that during muscular exercise or a state of anger or fright contractions of the spleen occur with an outpouring of additional red cells into the circulation. In addition, changes have been found in the chlorine and protein contents of the plasma and in the hæmoglobin content of whole blood during exercise in men even after splenectomy (Smirk—unpublished observations). The chlorine content of the plasma may vary independently of the plasma protein or the hæmoglobin content of whole blood either by movements of chlorine ions to and from the erythrocytes (the Hamburger phenomenon) or the tissues [this paper and unpublished observations]. Since the lymph may contain much protein the formation and storage in tissues or serous cavities of additional lymph as a result of water absorption would give rise to a fall in the plasma protein concentration even if the blood volume remained constant. It will be shown in a subsequent paper with Heller that this source of alteration in the plasma protein percentage is not purely theoretical. Again venous congestion of the part from which the blood sample is taken may alter the concentration of hæmoglobin and the distribution of chlorine between corpuscles and plasma; and even heating the source of the blood sample to obtain a more liberal flow may produce small alterations in man [Smirk, 1928 *a*].

It may be suggested that since exercise, emotional changes and many other factors may alter the concentration of various substances in the blood it will be well to avoid them by some such device as anæsthesia, decortication or decerebration.

The object, however, of this work is firstly to enquire whether it is indeed true that the tissues react rapidly and quantitatively to small changes in concentration of the blood in such a manner as to keep constant the blood volume, and secondly, to determine what relation, if any, there may be between blood concentration and normal water diuresis. The first object is of importance since if attained it may help to reveal whether the partition of water between blood and tissues is or is not mainly determined by physico-chemical changes. The second consideration should show whether in all animals the kidney is extremely sensitive to changes in blood concentration, or whether a biased view point has so far been obtained by considering mainly those animals where the renal response is most active.

Since most anæsthetic substances alter, usually diminishing, the urinary output, they are clearly unsuitable for the study of changes in blood concentration during the progress of a normal water diuresis. In

addition Hicks and I [1930] have shown directly on rabbits that the blood which became diluted after giving water by the alimentary tract was reconcentrated if chloretone or morphine were then administered. This perhaps indicates an altered distribution of water between blood and tissues under the influence of at least two narcotic drugs.

Although the decorticated or decerebrated preparations described by Fee [1929] gave responses to water administration as gauged by the urinary output, it does not appear safe to assume from this that the "pre-renal" distribution of water about the body, particularly its partition between the blood and tissues, must have remained normal. Especially is this so while many schools of thought emphasize the importance of the nervous system in determining water interchange and excretion.

For this reason it has been considered most desirable to work upon intact unanæsthetized animals and man and to eliminate the effects of struggling and emotion by means of controls in which the entire experimental procedure is repeated with the exception of water administration. Also it has been thought desirable to use many different substances as indices of blood dilution. In this paper are described only the experiments on rabbits.

Experimental procedure.

The rabbits received 4 p.c. of their body weight of warm water by stomach tube, their previous dietic state being controlled.

Samples of blood were taken without congestion, and as far as possible without the production of fright or struggling, from the marginal ear veins.

Estimations of the plasma protein were made by Kjeldahl's method, no correction being made for non-protein nitrogen. Hæmatocrit observations were made by a method previously described [Smirk, 1928 *b*]. The percentage solid substance in whole blood was determined by receiving a small quantity of blood into a weighing bottle and determining the loss on drying to a constant weight by heat in an oven with a steady temperature of 107° C. for 36 hours. Hæmoglobin changes were detected by preparing solutions of 0·1 cm. of the bloods in 25 c.c. of tap water and passing coal gas in order to convert hæmoglobin to carboxyhæmoglobin. Using one of the samples before water administration as the standard the changes in concentration were determined by colorimetry and were expressed as a percentage fall below the original concentration without determination of the actual amount of hæmoglobin. In some experiments the iron content of the blood, which depends almost solely on the hæmoglobin, was determined by a method already described [Smirk, 1927 *a*]. The plasma chlorides were determined by rapid destruction of the proteins with ammonium persulphate and nitric acid in the presence of silver nitrate followed by back titration with alcoholic ammonium thiocyanate in the presence of acetone as an end point intensifier [Smirk, 1928 *b*].

Samples of blood were shaken with air before analysing in order to correct any changes in the distribution of chlorine ions due to differences in the CO_2 contents of the samples.

Heparine was usually used as anticoagulant, but where only small samples of blood were taken for plasma protein analysis neutral potassium oxalate was employed to avoid errors due to the nitrogen content of heparine.

The amounts of blood removed were in all cases controlled by the removal of similar quantities of blood in the control animals, and the necessity of adopting this procedure was made evident.

Urine samples were obtained by expression.

Results.

The changes in blood concentration after water administration have been expressed as a percentage of the original concentration. In other words, the initial blood concentration of any substance used as an index of blood dilution is called 100 p.c., and if the concentration of this substance falls to, say, 96 p.c. of this in the course of an experiment it is recorded simply as a 4 p.c. dilution. In all the charts the horizontal line represents the initial blood concentration—a line drawn vertically downwards from this represents a test in which the blood has become diluted: vertically upwards a test where the blood has been concentrated. Each vertical line, therefore, represents a separate experiment and the length of the line the degree of dilution or concentration.

(*a*) Control observations on the effect upon the haemoglobin concentration of removing 2 c.c. or 3 c.c. of blood.

In a 2-kilogram rabbit one would anticipate a blood volume of not less than 120 c.c., and theoretically the change in hæmoglobin percentage resulting from the removal of 2 c.c. or 3 c.c. should not exceed 1·7 p.c. to 2·5 p.c. In actual practice this is not so. A second sample of blood removed 1½ hours later may show a dilution of as much as 8 p.c., usually about 3 p.c. or 4 p.c. It is unlikely that this finding depends on any methodical error, since estimations of the plasma protein and hæmatocrit observations are in agreement with the hæmoglobin determinations: and it will be seen from subsequent control experiments where only 0·5 c.c. to 0·8 c.c. of blood have been removed (Diagrams 1, 2, 4) that such

changes are not then observed. It is not proposed to pursue this matter further, but it may well be that compensatory adjustments of blood volume in response to small hæmorrhages are not accurate to 3 p.c.

(*b*) The effect of the administration of 4 p.c. of the body weight of water upon the haemoglobin concentration of a sample of blood taken $1\frac{1}{2}$ hours later together with control observations in which the experimental procedure was repeated: water administration excepted. See Diagram 1.

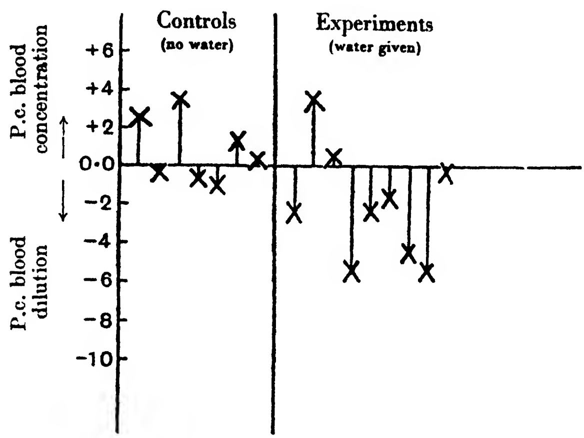

Diagram 1. Changes in hæmoglobin.

Diagrams 1–7 show the effect of water ingestion on the concentration of various blood constituents in the rabbit. Each vertical line represents a separate experiment. A fall of the vertical line below the base line represents a blood dilution.

Only 0·5 c.c. to 0·8 c.c. of blood were removed per sample and the full period of $1\frac{1}{2}$ hours was allowed to elapse before the second blood sample was taken in the control observations. The previous diet and treatment of the control animals was identical with that of animals receiving water and in no case were animals deprived of water before the experiments for a time long enough to cause appreciable blood concentration, the correction of which after water administration would simulate blood dilution. Rabbits whether on diets of oats and unlimited water or cabbage' and unlimited water showed dilution of the blood after the additional dose of water by stomach tube as contrasted with the absence of blood dilution in the controls where no additional water had been given.

It will be observed that the hæmoglobin concentration in the controls is not steady, but this might be expected from the presence of variations introduced by factors such as splenic contraction which have been referred to in the introduction. The average of all the control observations would suggest about 1 p.c. concentration of the blood which is approximately the same as the control observations for the plasma protein.

In one experiment where water has been given, a blood concentration was observed and this likewise may be due to variations in the amount of circulating hæmoglobin induced by the spleen. The remaining experiments suggest that there is a definite dilution of the blood as estimated by the hæmoglobin concentration.

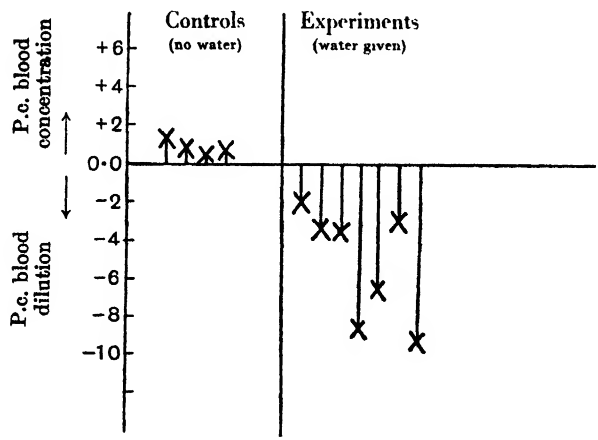

Diagram 2. Changes in plasma protein.

(*c*) The effect of the administration of 4 p.c. of the body weight of water upon the plasma protein concentration, together with control observations. See Diagram 2.

The procedure and precautions in these experiments have been exactly similar to those of the last series (Section (*b*)). The estimations of nitrogen by Kjeldahl's method were made in duplicate or triplicate on 0·1 c.c. of plasma each.

The concentration of protein tends to remain steady in the controls: being concentrated about 1 p.c. as would be expected from a urine loss which is not replaced by additional water given. It is probable that variations in the size of the spleen do not affect the protein concentration to the same degree as the hæmoglobin.

In contrast to the controls, animals receiving water showed in all cases a definite plasma dilution although the degree of dilution was variable.

(*d*) The effect of the administration of 4 p.c. of the body weight of water on the percentage of solid matter in whole blood. See Diagram 3.

Again the experimental procedures of Sections (*b*) and (*c*) have been repeated with the addition that a series of five observations were made on blood samples taken $\frac{1}{2}$ hour after water administration in addition to eleven observations at $1\frac{1}{2}$ hours. Further controls were considered

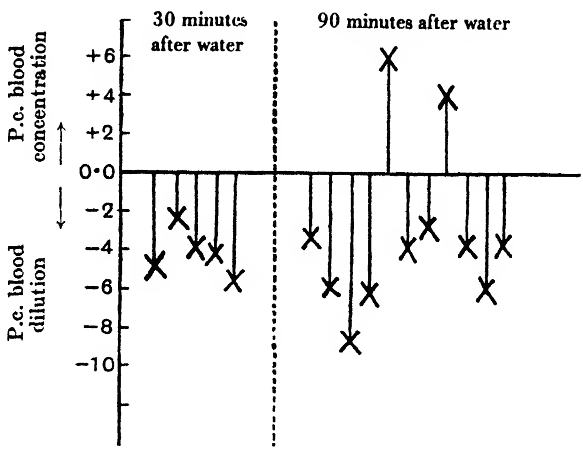

Diagram 3. Changes in the dried weight of whole blood.

unnecessary here since the percentage solid matter depends largely on the hæmoglobin and protein in which the absence of any important variation has been demonstrated.

Both at $\frac{1}{2}$ and $1\frac{1}{2}$ hours after giving water the blood was diluted. The two experiments in which a concentration of the blood was observed and some of the experiments where the dilution appears unusually great are probably explained by the splenic and other errors outlined in the introduction.

(*e*) The effect of the administration of 4 p.c. of the body weight of water on the percentage volume of red cells, together with control observations. See Diagram 4.

It will be observed that any difference there may be between the control animals and the animals that received water is much smaller

when the hæmatocrit readings are studied than in experiments where the hæmoglobin or plasma protein is used as an index of blood dilution. In order to study this in detail a series of results were collected together in which both hæmatocrit observations and hæmoglobin determinations were made on the same blood samples. These results are described in Section (*g*). For the moment it will suffice to state that the dilution estimated by the change in the percentage volume of corpuscles is as an average much less than the dilution estimated by the hæmoglobin percentage, the solid matter in blood or by the plasma protein. (See Diagrams 1, 2, 3, 4.)

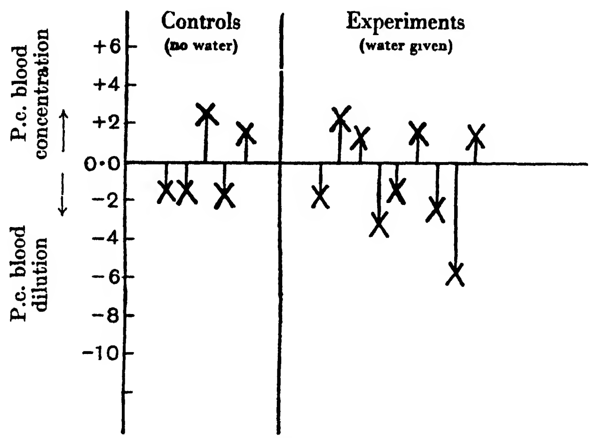

Diagram 4. Changes in the hæmatocrit.

(*f*) The effect of the administration of 4 p.c. of the body weight of water on the chloride content of plasma. See Diagram 5.

It will be observed that a slight fall in the plasma chlorine is the rule, and that this fall is less than the fall in plasma protein.

(*g*) A comparison of the degrees of blood dilution as determined by haematocrit observations and haemoglobin estimations on the same blood samples after the administration of 4 p.c. of the body weight of water. See Diagram 6.

Changes in the hæmoglobin percentage may be used as indications of the total amount of additional fluid which enters a given volume of blood, whereas the hæmatocrit readings inform us as to changes in the percentage volume of red cells; and therefore indicate the manner in which water is distributed between the plasma and the corpuscles: since upon this distribution depend changes in their proportional volumes.

In Diagram 6 are collected the results of experiments in which hæmatocrit observations were made and a dilution of the hæmoglobin was observed.

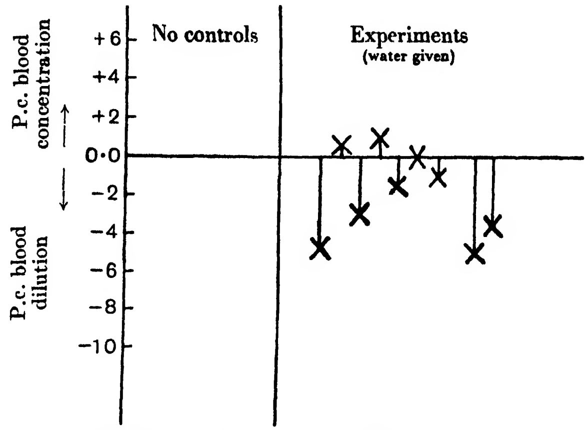

Diagram 5. Changes in the plasma chloride.

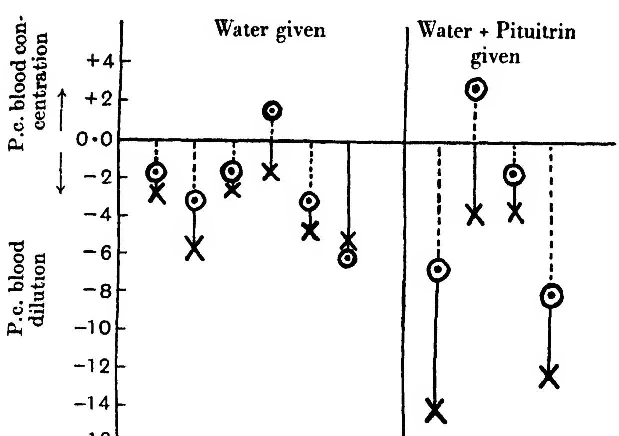

Diagram 6. Comparison of hæmatocrit and hæmoglobin dilutions in blood after water administration.

× Hæmoglobin dilution. ⊙ Hæmatocrit dilution.

In addition are included hæmatocrit and hæmoglobin determinations from four experiments in which the excretion of urine was prevented by pituitrin and a larger blood dilution resulted.

Of ten observations, each the average of two or three hæmoglobin determinations and six to eight hæmatocrit readings, nine showed that

the percentage blood dilution appeared greater when hæmoglobin was used as an index of dilution, and in the remaining experiment the two fell within experimental error. Of the nine results showing greater dilutions with the hæmoglobin two showed a slight concentration by the hæmatocrit. This may well be due to some such factor as splenic contraction, which by throwing additional blood into the circulation would tend to diminish the dilution effect of the water without greatly disturbing the relationship which has been described between the hæmatocrit and the hæmoglobin readings. In other words both points would be moved vertically upwards on the diagram.

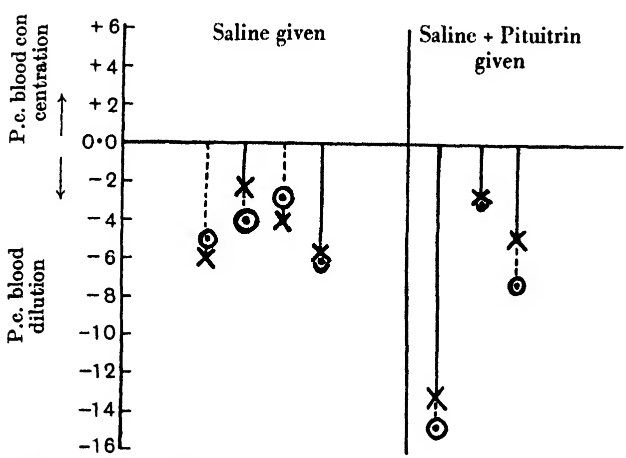

Diagram 7. Comparison of hæmatocrit and hæmoglobin dilutions in blood after 0·9 p.c. saline administration.

× Hæmoglobin dilution. ⊙ Hæmatocrit dilution.

One may briefly conclude from these observations that most of the entering water remains in the plasma but that some must enter the corpuscles and cause an increase in their average size.

(*h*) A comparison of the degrees of blood dilution as determined by haematocrit observations and haemoglobin estimations on the same blood samples after the administration of 4 p.c. of the body weight of 0·9 p.c. saline. See Diagram 7.

It was thought that a possible explanation of the distribution of added water between plasma and corpuscles which has been described in Section (*g*) might be, that the water enters the blood as hypotonic saline, or at least that adjustments take place which render the net

result an equivalent of this. Therefore it was decided to examine the distribution of the added water when isotonic saline was administered, since from *in vitro* experiments one would then expect to find the whole of the added fluid in the plasma. This anticipation was realized since the dilutions as estimated by the hæmoglobin and the hæmatocrit were then approximately equal, and this was especially evident when large blood dilutions were produced with the help of pituitrin.

Thus where water was given the average of all the results (including pituitrin experiments) gives a dilution of 5·5 p.c. for the hæmoglobin and 2·6 p.c. for the hæmatocrit, but where saline is given although the dilution is still 5·5 p.c. for the hæmoglobin it is 6·0 p.c. for the hæmatocrit.

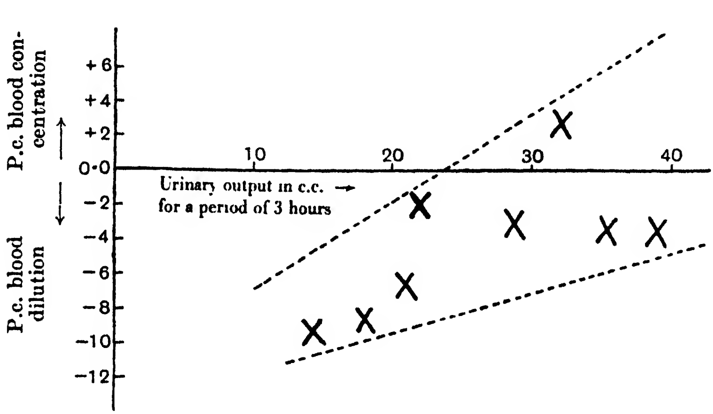

Diagram 8. The relation between the dilution of plasma proteins and diuresis (rabbit).

This can only be the case if where water was given a proportion of the water entered the corpuscles and where isotonic saline was given all the water detained in the blood remained in the plasma.

(*i*) The relationship between diuresis and the dilution of the plasma protein.

In Diagram 8 has been represented the relationship of the output of urine over the 3 hours following water administration to the dilution of the plasma protein expressed as a percentage. The abscissa represents the urinary output in the 3-hour period per kg. of the animal's body weight and the blood samples were taken shortly before and 1½ hours after water administration. It will be observed that as a rule the large blood dilutions are met in animals with a small diuresis and conversely the animals with a relatively large diuresis have the smaller blood dilu-

tion. It can at least be said that the diuresis over the full period of 3 hours is not proportional to the degree of plasma dilution as estimated by the plasma protein, and it would even seem probable that the cause of blood dilution may sometimes be the inadequacy of the renal response.

It may be suggested that one cannot fairly relate the urine output for 3 hours after the administration of water to the plasma composition at the mid-way point (*i.e.* 1½ hours after).

Similar charts have been constructed, however, for the period 1½ hours before the second blood sample and also for the 1½ hours afterward, and both show the same distribution of results. The 3-hour chart may, therefore, be given as being representative.

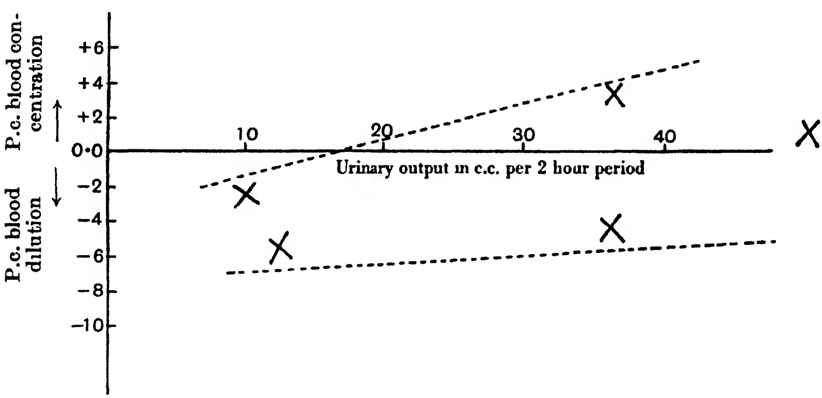

Diagram 9. The relation between the dilution of hæmoglobin and diuresis (rabbit).

(*j*) The relationship between diuresis and blood dilution as measured by the hæmoglobin of whole blood.

In precisely the same manner as in Section (*i*), Diagram 9 relates the hæmoglobin dilution at 1½ hours after water administration to the urinary output during the 2 hours after giving water. Similar relationships are obtained if the blood dilution is related to the output of urine during the ½ hour following the taking of the second blood sample or to the urinary output during the 1½ hours after giving water.

The chart shows that the degree of blood dilution does not determine the degree of diuresis, and again, as with the plasma protein, results are perhaps more compatible with the idea that it is the absence of an adequate renal response to water administration which may determine the blood dilution.

(*k*) The relationship between diuresis and the dilution of the total solid content of whole blood.

Diagram 10 which is constructed on the same principle as Diagrams 8 and 9 relates the dilution of the total solids 1½ hours after water administration to the output of urine in the second hour.

No relationship can be made out between dilution of the blood and diuresis. If the urinary output up to the time of taking the blood sample, or the urinary output for the full 3-hour period are studied, the same absence of relationship is observed.

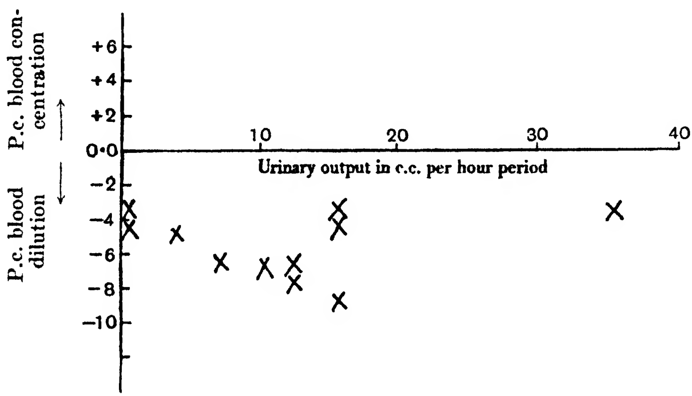

Diagram 10. The relation between the dilution of the total solids of blood and diuresis (rabbit).

Discussion.

By blood dilution is here meant a diminished concentration in blood of any of its main constituents: by general blood dilution a dilution of all of these or at least of such representative constituents as hæmoglobin, plasma proteins and plasma chloride.

From the consideration of certain fallacies outlined in the introduction it will be evident that changes in the concentration of any one substance in the blood do not justify the conclusion that there has been an alteration in the blood volume, since the salts, hæmoglobin and other proteins may vary independently. But dilution of two or three representative substances including those most abundant in blood must be regarded as very strong evidence of an increased water content of blood and strong evidence also of an increased blood volume.

The degree of the changes in blood concentration is always small

both relative to the quantities of water given and to the resulting diuresis, and the part played by methodical errors in analysis and sampling of blood must, therefore, receive careful consideration. As far as possible analytical errors have been controlled by duplicate and triplicate estimations, and sampling errors by avoidance of congestion and procedures liable to alter blood concentration.

The most important controls in my opinion are firstly the frequent comparisons with animals in which the whole experimental procedure is repeated with the exception of water administration, and secondly the use of several different indices of blood dilution involving as many different analytical processes.

Comparison of the results obtained in the animals which received water with the results in animals where no water was given make it, I think, certain that under the conditions of my experiment a general blood dilution was indeed the rule, and that this dilution was not occasioned by the removal of the first blood sample or by any other procedure than the giving of water.

It would not be difficult, however, to obtain results of this kind by depriving animals of water, thus producing a blood concentration which would be corrected only in those animals which subsequently received water, thus giving rise to an apparent blood dilution.

This, however, is not the explanation of the results since the rabbits were allowed free access to water up to or to within an hour of the experiment. The previous diet may be of importance since animals fed on cabbage have much larger outputs of urine than animals receiving oats, although the latter animals have free access to water. This also remains true when the rabbits have been deprived of food for 24 hours, and is not due to the additional water content of the cabbage. The general impression conveyed by my results is that animals fed on oats show slightly greater dilutions of the blood. The effect of a previous diet of cabbage upon diuresis persists some days even if oats have been given in the interval. It is not so likely therefore that the difference in urinary output operates through any alteration in the composition of the fluid absorbed from the alimentary tract. The chlorine contents of random samples of cabbage and oats (expressed as NaCl) were found to be respectively 0·298 and 0·057, and since a larger weight of cabbage can be eaten by a rabbit there is little doubt that on this diet its tissues and urine will be richer in salts.

Since, however, the animals were allowed water but deprived of food about 15 hours before the experiment it is not probable that the salt

content of the water in the alimentary canal prior to its absorption is greatly different under the two diets.

The animals used to study the relationships of protein and of the total solids of plasma to diuresis were both on uniform diets.

Inspection of Diagrams 1, 2, 3 and 5 reveals that although the same dose of water is given for each kilogram of the animals' body weight the degree of blood dilution differs from one animal to another to an extent which cannot be explained by chemical error. Although splenic contractions induced by fright or struggling might possibly explain the variability in the degree of blood dilution as estimated by the hæmoglobin and the total solids of whole blood it is unlikely that this would explain the varying degrees of protein dilution. The constancy of the protein content of plasma in the control observations also supports the contention that these results indeed represent varying degrees of change in the blood volume. Bayliss and Fee [1930] have suggested from their work on decerebrated dogs that the amount of additional water in plasma is such as would be expected if the extra water were uniformly distributed throughout the tissues. This does not appear to be the case in rabbits, and two reasons why a non-uniform distribution is to be expected at $1\frac{1}{2}$ hours after water administration in rabbits may be stated.

Firstly, whether and to what degree a blood dilution takes place depends partly upon a time factor operating through a system which may be represented diagrammatically as follows:

$$\begin{tikzcd}
 & \text{Alimentary canal} \arrow[d, shift left] & \\
\text{Tissues} \arrow[r, shift right] & \text{Plasma} \arrow[l, shift right] \arrow[u, dotted, shift left] \arrow[r] \arrow[d, shift right] & \text{Kidneys} \\
 & \text{Corpuscles} \arrow[u, shift right] &
\end{tikzcd}$$

It will be clear in such a system as the above that either all water entering the blood from the alimentary tract must be quantitatively and immediately removed by renal excretion or tissue absorption (very rarely by re-excretion into the lower alimentary tract) or a general blood dilution must result. The degree of a general blood dilution produced by a time lag in the disposal of water is not specially likely to be such as would be obtained by the uniform distribution of added water in the body.

Secondly, it would appear from these results that we must consider the effect of the salt content of the entering water upon its distribution. It is highly probable that water entering the blood stream from the

alimentary canal has already become hypotonic saline or rapidly attains equilibrium with the tissues, the net result being the addition to the blood of hypotonic saline.

It will be evident from Diagrams 7 and 6 that when the administration of isotonic saline produces blood dilution the degrees of dilution as measured by the hæmoglobin and by hæmatocrit readings are equal; whereas if a blood dilution results from the administration of water the change in the hæmoglobin percentage is greater than the change in percentage cell volume.

The only reasonable interpretation of these findings is that isotonic saline remains in the plasma and does not penetrate the corpuscles to an appreciable degree, but water to which no salt is added outside the body is distributed between the corpuscles and the plasma, the larger part, however, entering the plasma. These relationships remain true when larger blood dilutions are produced with the aid of pituitrin which diminishes the formation of urine.

Thus averaging all the seven experiments using isotonic saline the hæmoglobin dilution is 5·5 p.c. and the average cell volume dilution is 6·0 p.c. When water is given the hæmoglobin dilution averages 5·49 p.c., but in contrast the dilution of the cell volume is only 2·6 p.c., showing that a proportion of water has entered the corpuscles and increased their size: thereby the relative volumes of plasma and corpuscles are not altered to the same degree as is the hæmoglobin percentage.

It would appear justifiable to conclude from this that the distribution between the plasma and the corpuscles of extra water added to the blood is at least partly dependent upon the concentration of salt dissolved therein. It is also likely that water either enters the blood as hypotonic saline or rapidly takes up salt from the tissues so that the final result is equivalent to this. Variations in the salt reserves might easily account in this way for differences in the distribution of added water between corpuscles and plasma.

Nor is it unlikely that the effective salt content of added fluid normally influences its distribution between plasma and tissues. Thus hypertonic saline will cause dilution of the blood and a withdrawal of fluid from tissues, and conversely it is likely that the degree or at least the rate of penetration of water into the tissues will depend upon the degree of hypotonicity.

Although the experiments were carried out on different animals it is significant that the dilution of plasma as estimated by the plasma proteins is greater than the dilution as estimated by the plasma chloride.

This must mean either that protein has left the blood or salt has entered it, but not in a sufficient amount to render the extra water isotonic.

Observations on man also suggest that movements of salt occur after water administration, but in the direction blood to tissues. It is desired to treat of this in a subsequent paper.

It will be clear from Diagrams 8, 9 and 10 that the degree of diuresis is not proportional to the degree of blood dilution of the substances under consideration, and that where any relationship exists, as would appear in Diagram 8 and to a lesser degree in Diagram 9, it suggests that dilution follows upon inadequate diuresis. It is certain that a good diuresis may be present with little or no general dilution of the blood, and in apparently normal rabbits a definite general blood dilution may be accompanied by a poor diuresis. It does not appear in this animal that diuresis is causally related to general blood dilution. This observation is not easy to reconcile with Cushny's theory that the composition of the blood is the determinant of urinary composition and diuresis.

On the other hand, the absence of parallelism between blood composition and urine formation is compatible with the production of increased urine, being due either to the mobilization of some diuretic substance from the tissues or to a diminished content of the blood in the hormone of the pituitary body [Verney, 1926, 1929].

SUMMARY.

1. The percentage of solid substance in whole blood, the hæmoglobin of whole blood, the percentage cell volume (by the hæmatocrit), the protein content of plasma and the chlorine content of plasma have been used to study changes in blood concentration.

2. In most experiments on rabbits each one of the above yields evidence of some degree of blood dilution when 40 c.c. of water per kilogram of body weight are administered, and it is concluded from this that both the water content of the blood and the blood volume have been increased.

3. The average degrees of dilution in similarly conducted experiments vary according to which substance is used as an index of dilution, and reasons for these differences are suggested. Thus:

Index of dilution	Degree of dilution
(1) Percentage solid substance in whole blood	3·5
(2) Hæmoglobin of whole blood	2·0
(3) Percentage cell volume	1·9
(4) Protein of plasma	5·2
(5) Chlorine of plasma	1·6

4. In individual rabbits the degrees of blood dilution as estimated by various constituents are not uniform and may be in excess of or less than would be expected from an equal partition of added water among the water-bearing tissues of the body.

5. The administration of water or water and pituitrin produces chiefly a dilution of the plasma, but the red cells also take up some water and are increased in size.

6. Blood dilution produced by 0·9 p.c. saline or saline + pituitrin is entirely a plasma dilution.

7. It is probable in rabbits that the dilution of blood following the alimentary absorption of water is in fact a dilution with hypotonic saline. It is likely that the effectual salt content of the added water determines its distribution between corpuscles and plasma, and may also affect the rate at which it can be taken up for temporary storage in the tissues.

8. The degree of hæmoglobin, plasma protein and total solid dilution has played no significant part in determining the urinary output during these experiments. On the contrary, it is probable that general dilution of the blood is partly determined by inadequacy of the rate of diuresis relative to the absorption rate from the alimentary canal.

I should like to thank Prof. Verney for his helpful criticism and Dr Heller for permission to include a few results from work performed in collaboration.

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THE RESPONSES OF THE BATRACHIAN ALIMENTARY CANAL TO AUTONOMIC DRUGS. *RANA* AND *BUFO* ARECOLINE[1].

By DAVID EPSTEIN.

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It has been shown in previous communications [Epstein, 1931 and 1932] that the whole alimentary canal of *Xenopus lævis* (the South African clawed toad) responds to arecoline and to pilocarpine (on the whole) with contraction, relaxation being produced by the subsequent addition of atropine. These responses taken in conjunction with other evidence led the author to conclude that the whole digestive tract of *Xenopus* is supplied by parasympathetic motor fibres.

The responses to electrical stimuli and drugs of the alimentary tissues of other genera of Amphibia, *e.g. Rana* and *Bufo*, vary considerably according to different workers. It appeared to be of interest therefore to re-investigate this question and to compare the results with those found in *Xenopus*.

Experimental method.

The method employed was similar to that introduced by Magnus. Excised portions of batrachian alimentary canal were suspended in baths containing 50 c.c. of Howell-Ringer solution (NaCl 0·7 p.c., KCl 0·03 p.c. and $CaCl_2$ 0·026 p.c.), air being bubbled through at a constant rate and the movements of the tissues recorded graphically by means of levers. For further details and a list of references a previous paper should be consulted [Epstein, 1931]. The alimentary tissues of *Rana fuscigula* and *Bufo regularis* were employed in most of the experiments. Rarely, those of *R. fasciata* were also used. After the work on these animals had been completed, an opportunity arose of extending the investigation to include the small and large intestine of *R. temporaria*.

[1] A preliminary account appeared in the *Proceedings of the Physiological Society*, December, 1930. *J. Physiol.* 71, 5 *P*.

Experimental results.

I. *Arecoline hydrochloride* (Merck) and *atropine sulphate.*

(*a*) *Œsophagus.* In all experiments arecoline (1 in 100,000 to 1 in 50,000) produced well-marked, sustained rises in tone of the isolated

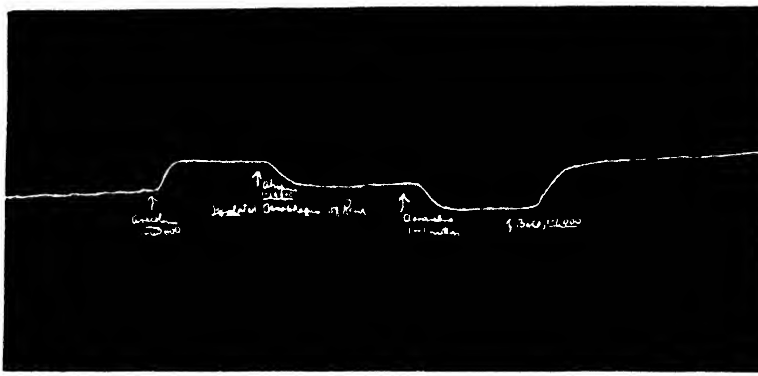

Fig. 1. Isolated œsophagus of *R. fuscigula.* Showing contraction with arecoline (1 in 40,000) and relaxation with atropine (1 in 50,000). Adrenaline (1 in 1,000,000) caused a further relaxation and barium chloride (1 in 1000) contraction. Note that here and in all subsequent figures an upward movement (↑) denotes contraction, a downward movement relaxation. All figures greatly reduced.

Fig 2. Œsophagus of *B. regularis.* Arecoline (1 in 50,000) caused a rise in tone and augmentation of the waves. Atropine (1 in 50,000) abolished these effects and relaxed the tissue below its previous level. Adrenaline (1 in 2,000,000) caused a further relaxation and $BaCl_2$ (1 in 2000) produced contraction.

œsophagus of *R. fuscigula* (Fig. 1) and of *B. regularis* (Fig. 2), which were followed by immediate relaxation on the addition of atropine (1 in 100,000).

(*b*) *Stomach.* In all experiments performed on the isolated stomach of *R. fuscigula* and *B. regularis* arecoline (1 in 50,000 to 1 in 25,000) again caused a definite rise in tone or (and) an augmentation of the automatic waves (Figs. 3 and 4). The subsequent addition of atropine (1 in 50,000)

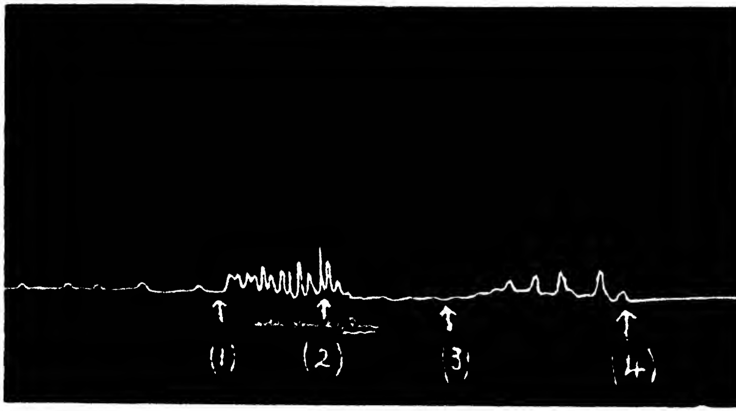

Fig. 3 Isolated stomach of *R. fuscigula*. Showing augmentation of the waves with (1) arecoline (1 in 40,000) which was antagonized by (2) atropine (1 in 50,000); (3) barium (1 in 1000) also augmented the waves, while (4) adrenaline (1 in 1,000,000) inhibited them.

Fig. 4. Stomach of *B. regularis*. Arecoline (1 in 50,000) caused a rise in tone and augmentation of the waves. Atropine (1 in 70,000) partially abolished these effects, but 1 in 30,000 was required to antagonize the effects completely. There was a prolonged latent period before the onset of the actions of the drugs.

caused inhibition of the waves or a lowering of tone or both these effects. In one experiment on *Rana* there was a transient relaxation preceding the rise in tone with arecoline. With the stomach of *Bufo* the latent period preceding the onset of the actions of the drugs was usually more prolonged than was the case in *Rana*.

(c) *Duodenum.* The results on the isolated duodenum of *Rana* differed from those seen in the œsophagus and stomach, for arecoline here produced relaxation (Fig. 5). The concentration of arecoline employed was usually 1 in 100,000, but the effect could be seen with a dilution of 1 in 400,000. If the arecoline solution was replaced fairly soon by fresh Ringer's solution, the tissue usually showed a rise in tone back to its previous level; the arecoline effect could then be repeated. If the arecoline solution was allowed to remain in the bath the tissue remained relaxed, but occasionally the effect gradually passed off.

Fig. 5. Duodenum of *R. fuscigula.* Arecoline (1 in 50,000) caused a lowering of tone, and a marked augmentation of the automatic waves occurred at the same time. Atropine (1 in 50,000) abolished the waves and caused the tissue to rise to its previous level. Adrenaline (1 in 1,000,000 and 1 in 300,000) had no effect, while barium caused contraction.

After arecoline had produced relaxation, the addition of atropine (1 in 50,000) usually antagonized the effect and caused the tissue to return to its previous level (Fig. 5). If the atropine had been applied first, it was able to prevent the arecoline effect. A concentration of at least 1 in 50,000 atropine was required for this purpose. In about one-fifth of the experiments atropine, having been applied after arecoline had produced relaxation of the intestine, failed to cause a rise in tone. In these cases the muscular tone of the tissue was probably very poor, so that, even though the atropine had antagonized the inhibitory effect of arecoline, the muscle was unable to contract and cause the tissue to rise to its previous level.

As the gut relaxed with arecoline the automatic waves were sometimes augmented (Fig. 5). This may simply have been a passive result of the

relaxation; but it sometimes seemed more like an active motor effect, for the first result of atropine applied at this stage was to abolish or diminish these waves and then cause the tissue to rise to its previous level (Fig. 5). On three occasions the duodenum relaxed spontaneously showing at the same time a similar augmentation of its automatic waves.

In two out of forty experiments on recently caught frogs there was a slight transient contraction preceding the relaxation with arecoline. In only one case did arecoline cause simple augmentation of the automatic waves of the duodenum, which were later inhibited by atropine.

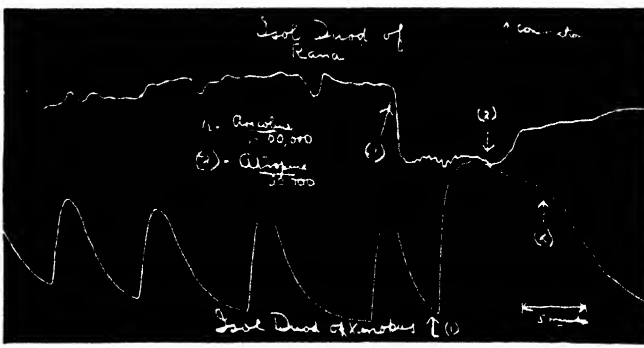

Fig. 6. Isolated duodenum of *Rana* (upper record) and of *Xenopus* (lower record). Both tissues suspended in the same bath. At arrow (1) (corresponding points on the two records as shown) arecoline (1 in 100,000) caused relaxation in the upper record and contraction in the lower one. At (2) atropine (1 in 50,000) antagonized both effects.

Care was taken not to include the distal end of the stomach, and the portion of gut immediately adjacent to the entrance of the bile duct into the intestine was generally employed.

The typical arecoline effects could be obtained only with the duodenum of r e c e n t l y c a u g h t frogs. The question of captivity will be discussed in a later paper, but it can be stated here that animals, which had been in captivity for only 2 or 3 days, proved most suitable, and that after 7–10 days the results were poor and even completely absent.

Similar results to those described above were obtained in the duodenum of *R. fasciata* and *R. temporaria* (Fig. 10).

Experiments performed on the duodenum of *B. regularis* with arecoline were indefinite. Out of eight experiments on recently caught animals four gave negative results (Fig. 11), two showed weak relaxation and two others gave slight contractions.

(In Fig. 6 the effect of arecoline on the duodenum of *R. fuscigula* has

been compared with its action on the duodenum of *X. laevis* (the South African clawed toad). Both tissues were suspended in the same bath. Arecoline relaxed the duodenum of *Rana*, but caused contraction of the same tissue in *Xenopus*. Both effects were antagonized by atropine.)

(*d*) *Jejunum and ileum.* Results identical with those in the duodenum were obtained, arecoline usually producing relaxation, although simple augmentation of the automatic waves was seen in isolated cases.

(*e*) *Rectum or large intestine.* Using freshly caught animals the isolated rectum of *R. fuscigula* and *R. fasciata* responded with relaxation to arecoline (1 in 100,000 to 1 in 50,000), atropine being able to prevent

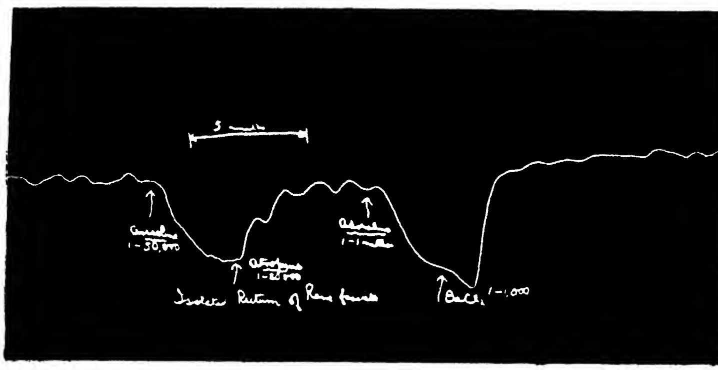

Fig. 7. Isolated rectum of *R. fasciata*. Arecoline (1 in 50,000) caused relaxation and atropine (1 in 50,000) abolished the effect. Adrenaline (1 in 1,000,000) produced relaxation and barium (1 in 1000) contraction.

or antagonize the effect (Fig. 7). On the other hand, the rectum of *R. temporaria* frequently responded with a weak contraction to arecoline.

Experiments carried out with arecoline on the rectum of *B. regularis* gave variable results. Thus, out of eight experiments, three were negative and five showed a relaxation which was unaffected by atropine.

(Figs. 8 and 9 show that arecoline produced relaxation of the rectum of *R. fuscigula*, but caused contraction of the rectum of *X. laevis*, while atropine antagonized both effects. Both tissues were suspended in the same bath.)

II. *Pilocarpine nitrate.*

Some experiments carried out with pilocarpine (1 in 100,000 to 1 in 30,000) on *Rana* caused contraction of the stomach, but the responses of the small and large intestine were indefinite and negligible.

III. *Physostigmine sulphate.*

Several experiments were performed on various alimentary tissues of *Rana* and *Bufo* with physostigmine (1 in 100,000 to 1 in 25,000), but the

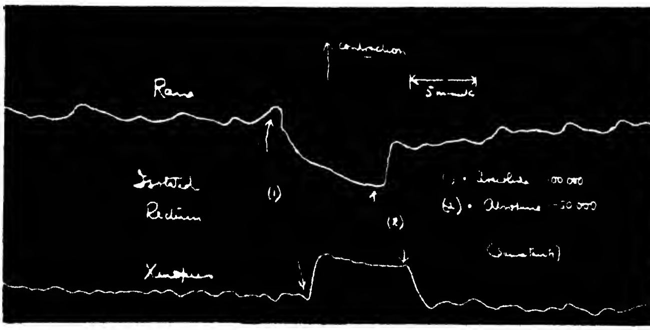

Fig. 8. Isolated rectum of *R. fuscigula* (upper record) and of *Xenopus* (lower record). Both tissues in same tank. At (1) arecoline (1 in 100,000) simultaneously caused relaxation of upper record and contraction of lower one. At (2) atropine (1 in 50,000) abolished both effects.

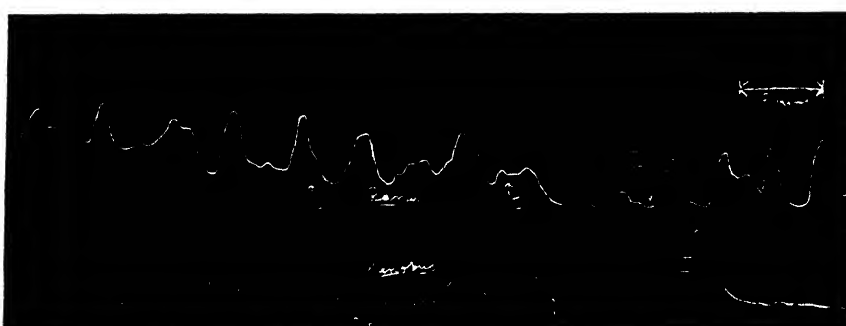

Fig. 9. Rectum of *R. fuscigula* (upper record) and of *Xenopus* (lower record). Both tissues in same tank. At I physostigmine (1 in 50,000) had no effect on either of the tissues. At II arecoline (1 in 70,000) caused relaxation of the upper tissue and contraction of the lower one. At III atropine (1 in 50,000) abolished the respective effects of arecoline on each of the tissues.

results were always negative (Fig. 9), except for one case on the duodenum, in which a slight relaxation was noted. Führer [1918] also failed to obtain any responses with physostigmine on the stomach of the frog.

IV. *Adrenaline.*

Several experiments were performed with adrenaline on each portion of the alimentary canal of *Rana* and *Bufo*, and relaxation of each tissue

Fig. 10. Isolated duodenum of *R. temporaria*. Nicotine (1 in 100,000) caused a contraction followed by a relaxation below the previous level. Several minutes later arecoline was also added to give a concentration of 1 in 100,000. The arecoline caused typical inhibition of the intestinal movements, which was antagonized by atropine (1 in 45,000). The level was raised slightly between the first and second parts of the record. Time intervals 30 min.

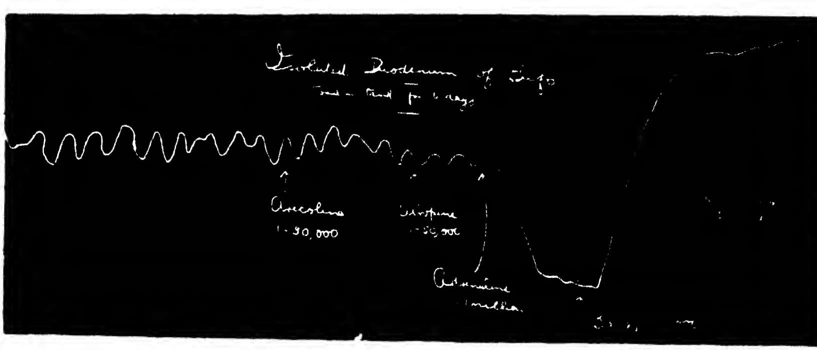

Fig. 11. Isolated duodenum of *B. regularis*. The effects of arecoline (1 in 50,000) and atropine (1 in 50,000) were negligible or nil; adrenaline (1 in 1,000,000) caused a very marked relaxation and barium chloride (1 in 1000) a powerful contraction. The animal had been caught only 6 days before.

was generally produced (Figs. 1, 2, 3, 7 and 11). In one experiment the isolated œsophagus of *Rana* gave a preliminary weak contraction. A concentration of 1 in 1,000,000 adrenaline was usually employed, but

definite relaxation could be produced with 1 in 100,000,000. In some of the experiments on the duodenum of *R. fuscigula* adrenaline failed to relax the tissue (Fig. 5).

V. *Barium chloride.*

Barium chloride in concentrations of 1 in 2000 to 1 in 1000 caused a strong contraction of each portion of the alimentary canal of *Rana* and *Bufo* (Figs. 1, 2, 3, 5, 7 and 11).

VI. *Nicotine.*

Nicotine tartrate and hydrochloride in concentrations of 1 in 200,000 to 1 in 25,000 were employed on the small and large intestine of *Rana.* The weaker strengths frequently caused contraction, which was followed sometimes by relaxation below the previous level (Fig. 10). The stronger concentrations usually caused relaxation only. Even after these large doses of nicotine had been applied, the subsequent addition of arecoline was still able to produce its typical effect, viz. relaxation of the duodenum and rectum of *R. fuscigula,* and the duodenum of *R. temporaria* (Fig. 10).

Discussion and conclusions.

Œsophagus and stomach.

It has been shown in the present series of experiments that weak concentrations of arecoline cause contraction of the œsophagus and stomach of *Rana* and *Bufo.* These effects can be antagonized by atropine but are unaffected by ergotamine. Further, the arecoline effects agree with the results seen when the parasympathetic nerves to the œsophagus and stomach are stimulated electrically. It would therefore seem justifiable to conclude that arecoline has stimulated the parasympathetic nerves supplying these organs and that the parasympathetic nerve supply here consists chiefly of motor or excitatory fibres.

Small and large intestine.

On the other hand, on the small and large intestine of *R. fuscigula* and *R. fasciata* and the small intestine of *R. temporaria,* arecoline (in the same weak concentrations) produces relaxation. This effect can be antagonized by atropine. Since the arecoline inhibition can still be produced after large, paralysing doses of nicotine (Fig. 10), it can be assumed that the arecoline action is not on the intrinsic ganglia of the gut, but must be situated peripheral to these structures, viz. on the nerve ends or the muscle tissue itself.

In attempting to localize more definitely the inhibitory effects of arecoline on the small and large intestine of *Rana* various hypotheses have been considered:

(1) Arecoline may be acting as a direct depressant of muscle tissue.

(2) The drug may have transferred its action from the parasympathetic to the sympathetic nerve endings, or

(3) it is possible that parasympathetic inhibitory fibres are present in the small and large intestine of *Rana* and that arecoline is acting upon them.

(1) *Muscle hypothesis.* The rapidity with which the arecoline relaxation comes on suggests nervous rather than muscular action. The effect has been seen with concentrations of arecoline as low as 1 in 400,000, and with such concentrations any muscular depressant effect will be negligible. Further, the action is antagonized by atropine, while concentrations of 1 in 2000 arecoline (which have a depressant action on muscle) are frequently unaffected by atropine. Also, the contraction with barium is unaltered by the previous application of weak strengths of arecoline. It would therefore seem justifiable to dismiss the muscular hypothesis and to seek an explanation for the arecoline effects in the other hypotheses mentioned above.

(2) *Sympathetic hypothesis.* The contractions of the œsophagus and stomach of *Rana* with arecoline have been attributed to an action of the drug on the parasympathetic motor system, while according to the hypothesis under consideration the relaxation of the small and large intestine by arecoline can be explained by claiming that the drug has transferred its site of action from the parasympathetic to the sympathetic nervous system. It has been shown by Cushny [1910] and others that a parasympathetic stimulant may in certain organs as a whole and under special circumstances act on the sympathetic system, but it seems unlikely that a drug would vary so greatly as to act on the parasympathetic in the upper half of the alimentary canal and to transfer its action to the sympathetic system in the lower half.

Further, the occasional failure of the duodenum of *R. fuscigula* to respond to adrenaline suggests the inactivation (and possibly the absence) of the sympathetic in these cases, and since arecoline is still able to relax the tissue in these experiments it would seem that the drug must be acting on some nervous mechanism other than the sympathetic, *i.e.* it is probably acting on the parasympathetic nervous system.

Finally, it has been demonstrated that when frogs of the genus *Rana* are kept in captivity for long periods, the arecoline relaxation of the

intestine fails to be obtained, but the relaxation with adrenaline at this time is frequently still present, which suggests that arecoline and adrenaline cannot be acting on the same sites, *i.e.* while adrenaline stimulates the inhibitory sympathetic, arecoline probably acts on the parasympathetic system.

(3) *Inhibitory parasympathetic hypothesis.* According to this hypothesis the actions of arecoline can be explained as follows: the œsophagus and stomach of *Rana* receive only (or chiefly) motor fibres from the parasympathetic system, and arecoline by stimulating their nerve endings produces contraction of these alimentary tissues. On the other hand, the small intestine (and the large intestine of certain species of *Rana*) can be regarded as receiving from the parasympathetic system inhibitory fibres chiefly, and it is by stimulating these fibres that arecoline causes relaxation of the tissues concerned. The points considered under the previous hypothesis also seem to indicate that arecoline has acted on parasympathetic inhibitory fibres in the intestine.

Previous workers have shown that parasympathetic inhibitory fibres are present in the œsophagus of the frog [Goltz], and in the stomach of the mammal [Langley, 1898; May and Elliott], frog [Hopf and Dixon] and turtle [Bercovitz and Rogers], so that their presence in the intestine of the frog seems possible.

The fact that a somewhat larger dose (1 in 50,000 at least) of atropine is required to paralyse the parasympathetic inhibitory fibres than the motor fibres (1 in 100,000 or less) of the frog's intestine, agrees with the findings of Langley [1898] and others in the case of the mammalian stomach.

The hypothesis under consideration is able to explain different phenomena as being due to a single drug acting on different parts of the same mechanism, and, until disproved, seems more acceptable than a theory which has to explain the actions by calling in the aid of two or more systems.

The preliminary contraction of the frog's intestine sometimes seen with small concentrations of nicotine may be due to an action of the drug on parasympathetic motor fibres present in the intestine but so few in number as to be usually masked by the action of the more powerful or more numerous inhibitory fibres. The occasional augmentation of the automatic waves, which accompanies the relaxation of the intestine produced by arecoline, could be explained in the same way. The parasympathetic nerve supply to the intestine of *Rana* can therefore be considered as containing an intermixture of motor and inhibitory fibres, the

inhibitory fibres, however, being greatly in the majority. Langley [1910] found that electrical stimulation of the sacral autonomic nerve fibres caused contraction of the rectum of *R. temporaria* accompanied by a preliminary elongation or relaxation, which also suggests an intermixture of fibres. Such intermixtures of motor and inhibitory fibres in parasympathetic nerves have been shown to occur in the vagus nerve supplying the cat's heart [Dale, Laidlaw and Symons], in the vagus nerve to the bronchioles of the cat [Brodie and Dixon] and in the pelvic nerve supplying the cat's urinary bladder [Langley, 1910*a*]. It is possible that such intermixtures of motor and inhibitory fibres in parasympathetic nerves occur more frequently than is at present suspected, and their demonstration may serve to explain results which at present are difficult to explain on a satisfactory basis.

From the results obtained by the application of various drugs (especially arecoline and atropine) to the alimentary canals of *Rana*, *Bufo* and *Xenopus* (for the results in *Xenopus* see a previous paper [Epstein, 1932] and also Figs. 6, 8 and 9) the author has concluded that the parasympathetic nerve supply to the digestive tracts of these genera of amphibia is as follows:

The œsophagus and stomach of *Rana*, *Xenopus* and *Bufo* receive from the parasympathetic nervous system motor fibres chiefly.

The small and large intestine: (*a*) in *Rana* the parasympathetic supply to the small intestine is inhibitory chiefly, while in the large intestine of some species it is again mainly inhibitory, but in others is chiefly motor; (*b*) in *Xenopus* the small and large intestine (like the œsophagus and stomach) receive mainly motor fibres from the parasympathetic (Figs. 6, 8 and 9); (*c*) while in the small and large intestine of *Bufo* the results have not been sufficiently decisive to allow of definite conclusions being drawn.

The results in Table I show the predominant type of the nerve supply from the parasympathetic system.

TABLE I. Parasympathetic nerve supply of batrachian alimentary canal.

Genus	Œsophagus	Stomach	Small intestine	Large intestine
Rana	Motor	Motor	Inhibitory	Inhibitory in some species, motor in others
Xenopus	Motor	Motor	Motor	Motor
Bufo	Motor	Motor	?	?

Summary.

The responses to autonomic drugs of the excised alimentary canals of various genera and species of Amphibia have been investigated.

From the results it has been concluded that in *Rana* the parasympathetic nerve supply to the œsophagus and stomach consists chiefly of motor fibres, while in the small intestine and (in certain species) in the large intestine too the parasympathetic fibres are mainly inhibitory.

In *Bufo* the parasympathetic supply to the œsophagus and stomach contains chiefly motor fibres also. No definite conclusions as to the parasympathetic supply to the small and large intestine of *Bufo* could be made.

The author wishes to express his indebtedness to Prof. J. W. C. Gunn of Cape Town University for the interest taken in this work, to Prof. J. A. Gunn of Oxford for the use of his laboratory to extend the investigations, and to Mr G. S. Grace, B.A., of Oxford, for assistance in carrying out some of the experiments on the intestine of *R. temporaria*.

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PHYSIOLOGICAL LEUCOCYTOSIS.

The variation in the leucocyte count during rest and exercise, and after the hypodermic injection of adrenaline.

By HAROLD ERIC MARTIN.

(*From the Manchester Royal Infirmary.*)

LITTLE is known concerning the normal variation of the leucocyte count. The following investigation was carried out to define the normal range of variation and the factors on which it depends as a preliminary to the study of Widal's hæmoclasic test for hepatic efficiency.

The Thoma-Zeiss method of counting leucocytes was found to be insufficiently accurate for this work, and the method adopted was a modification of that of Szabuniewicz [1928] and Shaw [1925]. The blood was withdrawn from a puncture on the dorsum of the finger, without squeezing, into a Thoma-Zeiss pipette. Three p.c. acetic acid was added to give a dilution of 1 in 20; the solution was shaken in a mechanical shaker, and counted in a Fuchs-Rosenthal counting chamber. Two chambers were filled and counted from each sample. The root mean square error of this method is 3·7 p.c. as compared with 17·5 p.c. in the Thoma-Zeiss method of enumerating leucocytes.

I. THE NORMAL VARIATION IN THE LEUCOCYTE COUNT DURING REST.

Hasselbalch and Heyerdahl [1908] and Ellermann and Erlandsen [1911] have both shown that changes of posture cause a variation in the leucocyte count. Recently Garrey [1929] has found that a recumbent position with complete physical and mental relaxation reduced the leucocyte count to a minimum which, in the eighty subjects investigated, lay between 5000 and 6000.

It was therefore deemed advisable to keep the patients recumbent and in a condition of absolute rest throughout the course of the experiments. For this reason convalescent patients without any organic lesion were selected as being more easily induced to lie quietly than healthy persons.

The patients, with one exception, were confined to bed during the whole of the day of the experiment. They were allowed light diet, being fed at 5.30 a.m., 12.30 p.m., and 3.30 p.m. Milk was given at 9.0 a.m. and 6.30 p.m. At 4.30 p.m. they were allowed to sit up and wash themselves. Six samples were taken from each patient. The times of sampling were spaced out between the hours of 10.0 a.m. and 8.0 p.m., the period between noon and 2.0 p.m. being avoided, owing to the possibility of post-prandial leucocytosis occurring during this period (Table I).

TABLE I. The variations in the leucocyte count during physiological rest. Two typical cases selected from a series of 12.

	Leucocytes per c.mm. blood	
Time	Case 5	Case 9
11 a.m.	5,000	12,200
12 noon	6,100	13,100
3 p.m.	4,600	—
4 ,,	—	12,300
5 ,,	5,800	12,900
6 ,,	5,500	13,600
7 ,,	5,800	13,500

The maximum and minimum counts for each case, together with their difference, are given in Table II.

TABLE II.

Case	Maximum	Minimum	Difference
1	8,500	6,400	2,100
2	7,200	6,200	1,000
3	13,200	10,100	3,100
4	7,800	7,300	500
5	6,100	4,600	1,500
6	11,200	8,100	3,100
7	6,400	5,500	900
8	6,500	5,400	1,100
9	13,600	12,200	1,400
10	10,000	8,400	1,600
11	8,700	5,600	3,100
12	7,600	5,900	1,700

Analysis of the results obtained.

The difference between the maximum and minimum counts in *Cases Nos.* 2, 4, 7 *and* 8 are relatively small, being 1000, 500, 900 and 1100 respectively. No conclusion is possible from *Case* 2, since the readings were not continued into the evening, the period when the maximum count is frequently obtained. All four patients were constantly quiet and placidly lay on their backs without varying their position or talking.

Case No. 1 was asleep at 2.30 p.m. and had to be wakened, and for the remainder of the day she was quite vivacious. Her count was at a minimum at 2.30 p.m. and steadily rose during the evening.

Case No. 3 was found sitting up at 4.0 p.m. During the latter part of the evening she was wide awake and reading. Her curve showed a peak at 4.0 p.m., and a considerable rise during the evening.

Case No. 6 was allowed up in the early morning, going to bed about 8.0 a.m. She went to sleep in the late afternoon. Her temperature chart showed a slight evening fall on the day of the experiment. Her leucocyte count was at a maximum in the morning and fell progressively through the day.

Cases Nos. 1, 3, 4, 5, 9, 10 *and* 11 showed a definite rise, and *Cases Nos.* 7, 8 *and* 12 a doubtful rise between 4.0 p.m. and 8.0 p.m. *Case* 6 alone shows a definite fall, and this has already been discussed. There is a definite fall between 2.0 p.m. and 4.0 p.m. in 75 p.c. of the cases (Nos. 1, 2, 3, 4, 5, 8, 9, 10), the minimum reading most frequently occurring during this period.

An observation of ward routine indicated that the early afternoon was the quietest part of the day. During this time the ward was run with a minimum staff, and many of the patients were asleep. In the late afternoon and early evening, however, the patients were washed and the beds were made.

Thus it will be seen that the leucocyte counts are at a minimum during the period when the patients' condition approximates most nearly to complete physiological repose and tend to rise with increase in the mental and physical activity of the patients. The leucocyte count, like temperature, tends to rise in the late afternoon and early evening, this being regarded physiologically as the most active part of the day.

In the observation of an afternoon rise, the work of both Sabin and co-workers [1925] and Shaw [1927] was confirmed. There is nothing in the above results to suggest the presence of the larger hourly oscillations observed by these workers. Since these individual oscillations were almost as great as the afternoon rise one would have expected them, had they occurred in the present investigation, to have caused considerable irregularity in the curves. Although the counts, in this work, were taken at longer time intervals than those of Sabin and Shaw, the irregular periods at which the readings were taken, and the larger number of cases investigated, preclude the possibility that the curve might be smooth, because each time it crossed the hypothetical oscillatory curve the latter was in the same phase.

In this section the diurnal variations in the leucocyte count have been investigated and the results obtained indicate that these variations depended upon the general activity of the subject.

It was decided next to determine the effects of exercise upon the leucocyte count.

II. The effect of exercise on the leucocyte count.

The following experiments were carried out upon apparently healthy female massage students aged 18–20 years. Before the beginning of each experiment the subjects were rested for a period of half an hour. Severe gymnastic exercises involving the muscles of the legs and trunk were used. These were carried out under the supervision of an instructress, and as far as possible they were maintained at the same degree of severity throughout the experiments. In order to prevent any error in the count due to congestion of the hands, the use of the arms was avoided.

In *Cases* 13–24 (Tables III and IV) a blood sample was taken from the resting patient, 5 min. exercise was performed and a second sample of blood taken immediately afterwards.

TABLE III. In *Cases* 13–18 exercise was carried out for 5 min. *Case* 13 is given as representative of this series.

	Min. after beginning of experiment			
	0		5	
	p.c.	Total	p.c.	Total
Case 13				
Polymorphonuclears	60·4	3624	56·50	4916
Lymphocytes	32·6	1956	39·25	3415
Large mononuclears	5·8	348	3·50	305
Eosinophils	0·8	48	0·75	65
Basophils	0·4	24	Nil	Nil
Total		6000		8700

TABLE IV. In experiments 19–24 exercise was carried out for 2½ min., a blood sample was taken as rapidly as possible and a further 2½ min. exercise performed. Case 19 is representative.

	Min. after beginning of experiment					
	0		2·5		5	
Case 19	p.c.	Total	p.c.	Total	p.c.	Total
Polymorphonuclears	55·75	5,129	55·0	5,775	52·25	6,113
Lymphocytes	33·50	3,082	34·75	3,649	35·75	4,183
Large mononuclears	6·50	598	8·50	893	7·50	878
Eosinophils	3·75	345	1·50	158	4·50	527
Basophils	0·50	46	0·25	26	Nil	—
Total		9,200		10,500		11,700

TABLE V. In the following cases: Exercise during first 5 min. Subject resting between 5 and 35 min. Splenectomy had been performed on Case 26, four years previously, for a cystic tumour of the spleen.

Cases	Min. from beginning of experiment: 0		5		35	
	p.c.	Total	p.c.	Total	p.c.	Total
25. Polymorphonuclears	67·50	4,815	62·50	5,316	67·00	4,606
Lymphocytes	21·00	1,498	25·00	2,126	25·00	1,718
Large mononuclears	9·00	642	9·00	765	7·00	481
Eosinophils	2·00	142	3·00	255	1·00	69
Basophils	0·50	36	0·50	43	Nil	—
Total		7,133		8,505		6,874
26. Polymorphonuclears	60·50	8,157	56·3	9,443	68·50	9,494
Lymphocytes	35·25	4,752	39·0	6,541	27·75	3,846
Large mononuclears	3·75	506	4·2	704	3·00	416
Eosinophils	0·25	34	0·3	50	0·25	35
Basophils	0·25	34	0·2	34	0·50	69
Total		13,482		16,772		13,860

TABLE VI. In the following cases: Exercise during first 5 min. and between 35 and 40 min. Subject resting between 5 and 35 min. and between 40 and 70 min.

Cases	Min. from beginning of experiment: 0		5		35		40		70	
	p.c.	Total	p.c.	Total	p.c.	Total	p.c.	Total	p.c.	Total
27. Polymorphonuclears	70·25	6,688	60·00	6,850	77·00	6,409	72·00	8,719	—	—
Lymphocytes	23·75	2,261	37·25	4,253	19·25	1,602	26·00	3,149	—	—
Large mononuclears	4·00	381	2·50	285	3·25	270	2·00	242	—	—
Eosinophils	1·75	166	0·25	29	0·50	42	Nil	—	—	—
Basophils	0·25	24	Nil	—	Nil	—	Nil	—	—	—
Total		9,520		11,417		8,323		12,110		—
28. Polymorphonuclears	68·50	4,397	43·25	3,851	57·00	3,140	55·50	4,596	—	—
Lymphocytes	26·75	1,717	53·00	4,719	38·50	2,121	36·50	3,022	—	—
Large mononuclears	3·50	225	2·50	223	3·00	165	6·00	497	—	—
Eosinophils	1·25	80	1·25	111	1·50	83	2·00	166	—	—
Basophils	Nil	—	Nil	—	Nil	—	Nil	—	—	—
Total		6,419		8,904		5,509		8,281		—
29. Polymorphonuclears	76·00	5,028	57·00	5,506	65·00	4,555	53·00	5,777	69·25	5,061
Lymphocytes	20·50	1,356	33·50	3,236	30·0	2,102	40·50	4,414	27·25	1,991
Large mononuclears	2·00	132	7·50	725	4·0	280	6·00	654	2·75	201
Eosinophils	1·50	99	2·00	193	1·0	70	0·25	27	0·75	55
Basophils	Nil	—	Nil	—	Nil	—	0·25	27	Nil	—
Total		6,615		9,660		7,007		10,899		7,308
30. Polymorphonuclears	52·50	3,513	50·00	4,449	54·25	3,752	62·50	4,987	52·00	3,724
Lymphocytes	43·50	2,911	44·75	3,981	41·75	2,887	31·75	2,534	46·00	3,294
Large mononuclears	3·25	218	3·75	333	3·50	242	4·75	379	1·00	71
Eosinophils	0·75	50	0·75	67	0·50	35	1·00	80	0·50	36
Basophils	Nil	—	0·75	67	Nil	—	Nil	—	0·50	36
Total		6,692		8,897		6,916		7,980		7,161

In *Cases* 25 *and* 26 (Table V) the subject was then rested in the sitting posture for 30 min. and a third sample of blood taken 35 min. after the beginning of the experiment.

In *Cases* 27 *and* 28 (Table VI) further exercises were performed between 35 and 40 min. from the beginning, a fourth sample being taken at 40 min., and in *Cases* 29 *and* 30 a fifth sample was taken after a second rest of 30 min.

To determine whether the leucocytes steadily increased throughout the exercise a series of experiments (*Cases* 19–24) was carried out in which a count was made after 2½ min. exercises and the exercise immediately continued for a further 2½ min., a second count being then taken.

The differential counts in all the experiments are based upon the enumeration of 400–600 cells. In Table VII is given the actual increase

TABLE VII. Summary. Effect of exercise on the leucocyte count.

Cases	Total leucocytes increase		Polymorphonuclears increase		Lymphocytes increase	
	Actual	p.c.	Actual	p.c.	Actual	p.c.
13	2700	45	1300	36	1500	75
14	– 1000	– 7	– 500	– 6	– 700	– 16
15	2200	23	100	2	1900	71
16	3700	41	1800	35	1800	59
17	1200	17	400	9	300	18
18	3000	33	1100	20	1600	54
19	2500	27	1100	21	1100	36
20	1700	16	45	1	1900	86
21	2500	28	200	4	2100	82
22	3300	31	1000	14	1000	47
23	2300	35	900	20	1400	76
24	1300	22	– 30	– 1	1300	67
25	1400	19	500	10	600	42
26	3300	25	1300	16	1800	38
27 1st exercise	1900	19	200	2	2000	88
2nd ,,	3800	45	2300	36	1500	97
28 1st ,,	2500	39	– 500	– 12	3000	175
2nd ,,	2800	50	1500	46	900	42
29. 1st ,,	3100	48	500	12	1900	138
2nd ,,	3900	55	1200	26	2300	110
30. 1st ,,	2200	33	900	27	1100	37
2nd ,,	1100	15	1200	33	– 400	– 12

per c.mm. and also the percentage increase of the total leucocytes, polymorphonuclear leucocytes and lymphocytes. In calculating the percentage values the actual number of each type of cell in one c.mm. of blood, before the exercise, was taken as 100 p.c.

To elucidate further the source of the polymorphonuclear leucocytes Schilling counts were performed in a selection of the cases (Table VIII).

TABLE VIII.

	Myelocytes		Metamyelocytes: Juveniles		Metamyelocytes: "Band" forms		Segmented polymorphonuclears	
Sample	p.c.	No. per c.mm.	p.c	No. per c.mm.	p.c.	No. per c.mm.	p.c.	No. per c.mm.
Case 13								
First	—		—	—	4·2	252	56·2	3372
Second	—	–	–	—	7·0	609	49·5	4307
Case 16								
First	—	-	0·5	45	1·5	135	54·5	4905
Second	–	—	0 25	32	4·75	603	49·25	6255
Case 18								
First	—	---	—	–	7 0	630	50·5	4545
Second	—	--	1·5	180	6·75	810	43·75	5250
Case 26								
First	—	–	—	--	3·25	439	57·25	7729
Second	-	—	—	-	3·5	588	53·0	8904
Case 27								
Third	—	--	—	-	2 5	208	74·5	6183
Fourth	–	–	..	—	2·0	242	70·0	8470

Discussion.

In all the experiments except *Case* 14 there was a rise in the total leucocyte count after 5 min. exercise varying from 15·75 to 48·0 p.e. and averaging 27·4 p.c.

Case 14 showed a fall in the total white cells, polymorphonuclears and lymphocytes and a rise in the large mononuclears. The subject was very stout, and mentally and physically lethargic. On inquiry it was found that her condition had been diagnosed as one of thyroid deficiency, and treated intermittently since childhood with thyroid extract. The treatment had been discontinued for the 3 months preceding the experiment.

In *Cases* 25–30 the exercise was followed by 30 min. rest, after which the leucocytes had fallen to approximately the normal value. Further exercise in *Cases* 27–30 caused a second rise which was sometimes greater, sometimes less than the first. During a second 30 min. rest in *Cases* 29 *and* 30 the counts again returned to roughly the normal value.

All types of cell contributed to the rise in the total leucocyte count, but the lymphocytes showed the greatest relative increase. The polymorphonuclear leucocytes showed an average increase of 11·67 p.c. after the first exercise. In *Cases* 24 *and* 28 there was a fall of 0·81 and 12·5 p.c. respectively in the count of these cells. The former figure lies within the experimental error.

After the second exercise there was in all cases a more marked

increase of polymorphonuclear leucocytes averaging 35·18 p.c. The lymphocytes yielded an increase after the first exercise varying from 18·4 to 175 p.c. and averaging 65 p.c. During the second exercise there was an increase in lymphocytes in all except *Case* 30. In this the lymphocytes continued to fall at approximately the same rate as they had been doing during the preceding rest period. It seemed as though the mechanism which caused the liberation of lymphocytes had been exhausted during the first exercise. It was discovered that the subject had been suffering from amenorrhœa and nervous instability for the preceding six months. Thyroid deficiency was diagnosed, and thyroid extract was being administered at the time of the experiment.

There was an increase during exercise of large mononuclears in fourteen and of the eosinophils in thirteen of the total eighteen cases examined. Owing to the large experimental error resulting from the small numbers of these cells counted, it was not possible to obtain a numerical estimate of their increase.

Case 26 was a splenectomized patient who reacted to exercise in exactly the same way as a normal subject: the observed leucocytosis is therefore not due to splenic contraction.

As the relative numbers of the different type of leucocytes vary during exercise, the observed leucocytosis cannot primarily be due to the concentration of the blood cells resulting from the passage of blood plasma into the tissue, or to the washing out into the blood stream of cells which have been trapped in quiescent capillaries.

In order to determine whether the leucocyte increase during exercise was due to the passage into the blood stream of immature cells from the bone marrow, Schilling counts have been carried out on selected cases, and the results are given in Table VIII. The increase in the polymorphonuclears was made up mainly of the mature leucocytes, but there was a small increase in the "band" forms, which indicates that the cells were probably liberated from the bone marrow. Since exercise is a normal physiological condition, a large influx of immature cells into the circulation is hardly to be expected, as they are rarely present in normal blood.

In Table IX is given the increase in total leucocytes, polymorphonuclears and lymphocytes during the first and second halves of a 5 min. period of exercise. In all the experiments except No. 22 the increase of all these cells was more marked during the first than during the second half of the exercise. In *Case* 22 the increase of the total leucocytes and polymorphonuclears was much greater during the latter half of the exercise. During the latter half of the exercise there was a fall in the

TABLE IX. Leucocyte counts taken in the middle of a period of 5 min. exercise.

I. = Increase during first half of exercise.
II = Increase during second half of exercise.

Case		Total leucocytes increase		Polymorphonuclears increase		Lymphocytes increase	
		Actual	p.c.	Actual	p.c	Actual	p.c.
19.	I.	1301	14·80	646	12·6	557	18·1
	II.	1200	11·9	338	5·88	534	14·65
20.	I.	2501	23·2	1048	13·4	1664	77·2
	II.	- 801	- 6·02	- 1003	- 11·35	+ 207	5·4
21.	I.	2301	26·25	273	4·87	1872	72·0
	II.	100	0·9	- 63	- 1·05	250	5·6
22.	I.	898	8·4	21	26·8	613	28·7
	II.	2402	20·7	2011	25·6	397	14·4
23.	I.	2200	33·3	484	9·94	1399	76·2
	II.	100	11·35	412	8·4	- 8	- 0·25
24.	I.	900	15·00	120	36·1	793	40·7
	II.	400	58·0	- 147	- 42·6	506	18·45

total leucocytes in *Case* 20, a fall in the polymorphonuclears in *Cases* 20, 21 *and* 24 and a fall in the lymphocytes in *Case* 23.

In spite of every effort made to keep the severity of the exercise uniform during the whole of the experiment, it is probable that the subject tends to get tired and unconsciously to put less energy into the exercise during the latter part of the experiment. This is a possible explanation of the diminished increase in the leucocyte count as the exercise proceeds.

III. VARIATIONS IN THE LEUCOCYTE COUNT FOLLOWING THE HYPODERMIC INJECTION OF ADRENALINE.

In the previous section it has been shown that exercise causes a rise in the leucocyte count of the blood with a relative lymphocytosis. A leucocytosis following emotional disturbances has been demonstrated by Menkin [1928] and Barcroft [1930] in animals, and by Garrey [1929] in human beings.

As it is known that adrenaline is secreted into the blood during both excitement and exercise, it seemed possible that the above phenomena might be due to adrenaline causing contraction of the spleen and lymph glands of the body. It was therefore decided to investigate the effect of the subcutaneous injection of adrenaline on the leucocyte count.

Historical summary.

Very little work appears to have been done on the effect of adrenaline upon the leucocytes.

Camus and Pagniez [1908], in a paper on the relationship of arterial pressure and blood cells, quoted an experiment by Loeper and Crauzon, in which the injection of adrenaline was found to produce a rise in blood-pressure followed by a leucocytosis. The experiment was not repeated by Camus and Pagniez and the reference to the original paper was not given.

Yang [1928] investigated the influence of the hypodermic injection of adrenaline upon the spleen and upon the blood picture in four cases of splenomegaly, two cases after splenectomy and two normal subjects. He observed splenic contraction and a leucocytosis in all cases. The cases with splenomegaly showed no change in the differential count, the splenectomized cases yielded a marked, and the normals a slight leucocytosis. The normal cases were not well chosen: one was suffering from a considerable neutrophil leucocytosis due to a septic infection, and in all cases the different types of cells were left as percentages and not worked out as the actual number per c.mm.

Experimental.

In this work a 1 in 1000 solution of adrenaline chloride prepared by Messrs Parke, Davis and Co. was used. The solution was always obtained from a freshly opened bottle of a recently prepared batch. All the investigations were carried out on convalescent patients, who were kept recumbent in bed. No food was allowed during the experiment. The blood samples were taken from the hand opposite to the arm into which the adrenaline had been injected. The cell-counting technique was the same as that employed in the work on the leucocytosis caused by exercise. Blood samples were taken immediately before, and at specified time intervals after the hypodermic injection of the adrenaline.

Fourteen cases were examined and the results obtained are given in Table X. *Cases* 8 *and* 13 were subjects upon whom a splenectomy had been performed. Schilling counts were carried out on a selection of cases (Table XI).

In order to make sure that the results obtained were due to the adrenaline injected, and not to adrenaline secreted by the patient in the excitement of the experiment, a series of control experiments was performed in which distilled water was injected instead of adrenaline (Table XII).

TABLE X. The effect of the hypodermic injection of adrenaline on the blood count. The first count was taken immediately before the injection. The following are representative counts selected from a series of fourteen cases.

Min. after injection	Total leucocytes per c.mm.	Polymorphs		Lymphocytes		L. mononuclears		Eosinophils		Basophils	
		p.c.	Total	p.c.	Total	p.c.	Total	p.c.	Total	p.c.	Total
Case 1											
0	9,000	70·5	6,350	23·16	2,080	4·5	405	1·83	165	—	—
10	8,100	55·5	4,496	39·5	3,200	3·25	263	1·25	101	0·5	40
20	13,300	53·6	7,129	40·5	5,387	3·83	409	1·33	173	0·5	79·8
40	13,000	66·8	8,680	27·3	3,549	3·83	498	1·6	208	0·3	43
Case 3											
0	4,600	58·3	2,682	33·3	1,532	4·0	184	3·0	138	0·3	13·8
10	7,100	49·5	3,515	45·0	3,195	4·25	302	1·25	88	—	—
20	8,500	56·0	4,760	38·0	3,230	4·0	340	2·0	170	—	—
40	13,800	49·75	6,865	42·75	5,899	6·5	897	0·75	104	0·25	37·5

TABLE XI.

Sample	Myelocytes		Metamyelocytes: Juvenile		Metamyelocytes: Band forms		Segmented polymorphonuclears	
	p.c.	Cells per c.mm.	p.c.	Cells per c.mm.	p.c.	Cells per c.mm.	p.c.	Cells per c.mm.
Case 3								
First	—	—	—	—	3·7	170	54·6	2512
Fourth	—	—	—	—	4·85	669	44·9	6196
Case 4								
First	0·4	29	0·4	29	21·6	1555	44·6	3211
Second	—	—	—	—	22·8	3032	31·0	4123
Case 5								
First	—	—	—	—	9·25	740	43·75	3500
Fourth	—	—	—	—	10·3	1085	42·6	4479
Case 7								
First	—	—	—	—	19·50	2184	57·75	6468
Third	—	—	—	—	13·6	2040	54·4	8160
Case 9								
First	—	—	—	—	3 0	201	63·0	4221
Third	—	—	—	—	4·33	489	48·42	5472
Case 11								
First	—	—	—	—	22·5	1148	32·75	1670
Second	—	—	0·25	17	18·50	1277	31·00	2139

In Table XIII is given the maximum observed increase per c.mm., and also the corresponding percentage increase of the total leucocytes, polymorphonuclear leucocytes and lymphocytes. In calculating the percentage values, the actual number of each type of cell in 1 c.mm. of blood before the exercise is taken as 100 p.c.

TABLE XII. A characteristic control experiment selected from a series in which distilled water was injected instead of adrenaline.

Min.	Total leuco-cytes	Polymorphs		Lymphocytes		L. mono-nuclears		Eosinophils		Basophils	
		p.c.	Total	p.c.	Total	p.c.	Total	p.c.	Total	p.c.	Total
Case 17											
0	6500	53·3	3470	39·6	2575	5·6	364	1·0	65	0·3	22
10	6400	51·5	3300	41·0	2630	6·5	416	1·0	64	—	—
20	6100	52·5	3200	44·0	2680	3·0	183	0·5	30	—	—
40	7600	54·0	4100	37·5	2850	7·5	570	1·0	76	—	—

TABLE XIII. The maximum observed increase in leucocytes after the hypodermic injection of adrenaline.

Case	Time after injection (min.)	Total leucocytes increase		Polymorphonuclears increase		Lymphocytes increase	
		Actual	p.c.	Actual	p.c.	Actual	p.c.
1	20	4300	48	780	12	3300	159
2	20	1200	10	340	4	900	68
3	40	9200	200	4200	156	4400	286
4	10	6100	85	2300	48	3000	148
5	40	2500	31	1300	31	1000	32
6	40	1700	27	1300	39	400	14
7	20	3800	34	1500	18	2300	141
8	20	6300	71	330	6	5000	204
9	20	4600	69	1500	35	2500	157
10	20	2300	24	- 30	- 0·5	2200	55
11	10	1800	35	600	22·8	1200	70
12	40	1600	28	300	10	1200	44
13	40	5900	23	3100	17	3100	97
14	60	7900	59	2400	27	5700	161

Discussion.

The hypodermic injection of adrenaline causes an increase in the total leucocyte count which reaches a maximum varying from 10 to 200 p.c. and averaging 53·1 p.c. in 10–60 min. after the injection. A comparison with the control cases (Table XII) shows that in all except *Cases*, 2, 5, 6, 10 *and* 12 the increase lies well outside the variation in the leucocyte count after the hypodermic injection of distilled water.

TABLE XIV. A comparison of the effects of exercise and adrenaline upon the leucocyte count.

	The average increase p.c.	
	After exercise	After the injection of adrenaline
Total leucocytes	27·4	53·1
Polymorphonuclears	11·7	30·5
Lymphocytes	65·0	116·0

There is an increase in the number of all types of cell, but it is most marked in the lymphocytes. The polymorphonuclears show an increase of up to 156 p.c. and averaging 30·5 p.c. In one case (10) there was a fall of 0·5 p.c. in the polymorphonuclears. Schilling counts show that while the main increase is due to the mature polymorphonuclear leucocytes, there is also a rise in the younger "band" forms. The increase on the lymphocytes varied from 14 to 286 p.c. and averaged 116·9 p.c. The large mononuclears, eosinophils and basophils are somewhat variable, but on the whole all these types of cell tend to show a rise. The splenectomized cases (8 and 13) reacted in exactly the same way as the normal subjects.

When the effect of exercise and the hypodermic injection of adrenaline upon the leucocyte count are compared, it will be found that they are very similar. In both cases all types of cell show an increase, while that of the lymphocytes is the most marked. A comparison of the increase in the total leucocytes, polymorphonuclears and lymphocytes after exercise and the injection of adrenaline is given in Table XIV, and it is seen that the ratio of the average percentage increase of the different types of cells is of the same order, the increase after the hypodermic injection of adrenaline being about double that after exercise. Since it is known that adrenaline is secreted during exercise, it seems probable that this is the cause of the leucocytosis following exercise.

The leucocytosis following exercise occurs immediately, while that following adrenaline hypodermically injected reaches its maximum 10–60 min. later. This is to be expected since adrenaline physiologically secreted passes directly into the general circulation, while adrenaline injected hypodermically only reaches the general circulation after a time interval which depends partly upon the vascularity of the tissue into which the adrenaline is placed, and partly upon the vaso-constrictor effect which the adrenaline exerts upon the peripheral vascular system.

The possible source of these leucocytes has next to be considered. The number of the circulating leucocytes may be increased by:

(1) Expulsion from the spleen.

(2) Dilatation of quiescent capillaries.

(3) Re-entry into the blood stream of cells adherent to the vessel walls.

(4) Concentration of the cells following the withdrawal of plasma from the blood.

(5) Expulsion from the lymphatic glands.

(6) Expulsion from the bone marrow.

(1) *Expulsion from the spleen.*

Barcroft [1926] has demonstrated in animals that the spleen contracts during exercise, and liberates red blood cells into the circulation. Schäfer and Moore [1896] and Yang [1928] have both shown that adrenaline causes splenic contraction. Viale and Bruno [1927] have suggested that the spleen regulates the number of circulating leucocytes. The effects on splenectomized patients of exercise, and the hypodermic injection of adrenaline have been examined, and do not differ from those on normal subjects. The leucocytosis cannot therefore be due to splenic contraction.

(2) *Dilatation of quiescent capillaries.*

Garrey [1929] has attributed the leucocytosis following exercise to the dilatation of quiescent capillaries by the increased cardiac activity, with the liberation of trapped leucocytes from them.

On this view, the passive dilatation of the capillaries should produce a leucocytosis. I have examined the effect on the leucocyte count of peripheral dilatation of the capillaries by means of hot baths, and of both peripheral and visceral dilatation by means of the inhalation of amyl nitrite. In neither case was a leucocytosis observed. One would expect the cells to be trapped in the capillaries in the same proportions as they occur in the circulating blood. In the experiments cited in this paper it has been found that lymphocytes predominate.

(3) *Re-entry into the blood stream of cells adherent to vessel walls.*

Ellermann and Erlandsen [1911] have advanced the theory that during exercise the increased cardiac activity causes the cells normally adherent to the blood vessel walls to be swept into the active blood stream. Krogh [1929] has found that there is some slight adhesion between the surface of the leucocytes and the endothelium of the blood vessels, but there is no evidence that the phenomenon is sufficiently marked to explain the relatively large leucocytosis observed after exercise. If this theory were true, one would expect a relative polymorphonuclear leucocytosis, since these cells show much more marked adhesive properties than the lymphocytes.

(4) *The concentration of blood cells following the withdrawal of plasma from the circulating blood.*

This theory has not been advanced previously, and it is difficult to prove, owing to the absence of an unvarying cellular element of the

blood that does not show numerical variations, and which could be taken as a standard. Since no two types of cell show the same rate of increase, the theory can at the best only play a subsidiary part in explaining the observed leucocytosis.

(5) *Expulsion from lymph glands.*

In the experiments on exercise and adrenaline it has been found that there is a predominant lymphocytosis. Since lymph glands contain smooth muscle, it occurred to me that the lymphocytosis might be due to its contraction under the influence of adrenaline with the resultant liberation of lymphocytes. The effect of adrenaline on isolated lymph glands was investigated. Lymph glands dissected from the mesentery of a dog were linked together in series, and immersed in a bath of Ringer's solution which was oxygenated and maintained at a temperature of 34° C. The lower end of the chain of glands was attached to the bottom of the bath, and the upper end was fastened to a straw lever recording on a smoked drum. On the addition of 0·05 c.c. of 1 in 1000 adrenaline chloride solution, the glands showed a contraction lasting about 7 min. The glands were washed *in situ*, and fresh Ringer's solution added. On adding more adrenaline the glands again contracted.

The lymph glands are to be regarded as the likeliest source of lymphocytes.

(6) *Expulsion from the bone marrow.*

Schilling counts performed before and after exercise, and before and after the hypodermic injection of adrenaline show that the increase in polymorphonuclear leucocytes is made up of both mature and immature cells, suggesting that the cells originate from the bone marrow. Adrenaline stimulates the general circulation by raising the blood-pressure and increasing the force and frequency of the heart, and the polymorphonuclears are probably washed out of the bone marrow in abnormally large numbers by the increased circulatory activity.

Summary.

1. The diurnal variation of the leucocyte count during rest has been examined and it has been shown:

(*a*) that the counts are steadiest and at a minimum when the subject's condition approximates most nearly to that of absolute physiological rest;

(*b*) that they rise with increase in the mental and physical activity of the subject, and

(*c*) that they tend to rise in the late afternoon and early evening, this being regarded as the period of greatest activity.

2. The effect of exercise on the peripheral blood was investigated. The total leucocyte count exhibited a rise varying from 15 to 48 p.c. of the resting value, and it returned to normal after about 30 min. rest. Further exercise then produced another rise in the count similar in type and degree to the first.

The rise in the total count was due to an increase in the number of all types of cell, but the lymphocytes showed the greatest increase.

3. The hypodermic injection of adrenaline is followed by a leucocytosis in which all types of cell show an increase, but that of the lymphocytes is most marked.

It is similar to the leucocytosis following exercise, and the theory has been advanced that the latter is produced by autogenously secreted adrenaline.

The cells which cause the leucocytosis arise from two sources.

The lymphocytes are in all probability derived from the lymph glands which contract under the influence of the adrenaline, and the granular cells are washed out of the bone marrow by the increased blood flow due to cardiac stimulation by the adrenaline.

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NORMAL RESPIRATION AND THE INFLUENCE OF THE VAGI.

By E. SHARPEY-SCHAFER (*Edinburgh*).

Normal respiration consists of inspiration and expiration without any pause, whether the respirations are slow or fast. In man the ratio of inspiration to expiration is approximately 3 : 4, but with individual variations. As is well known the rate varies considerably in different individuals, the variations being much greater than those of the pulse. The average rate in a normal man may be given at about sixteen a minute, but it is as high as twenty-four and as low as four. There are usually five to eight heart beats to each respiration. A tracing which has been reproduced in many text-books showing more than twenty heart beats to each respiration is obviously abnormal.

A slow pulse is not necessarily accompanied by slow respirations. Thus, in Fig. 1, from a man of 44, the pulse is 70 per minute, the respirations 9; in Fig. 2, from a lad of 16, the pulse is 44, the respirations 14; in Fig. 3, from a man of 36, the pulse is 68, the respirations 5.

Fig. 4 is from a dog, showing simultaneous tracings of respiration (*r.*), of aortic blood pressure (*ao.*), and of pulmonary blood-pressure (*p.*). There are five heart beats to each respiration. It will be noticed that, as in man, there is no pause between expiration and inspiration. This is true also of other animals. Under conditions in which the respiratory centre is depressed a definite pause may become apparent. Such conditions can be produced by drugs, especially anæsthetics in excess, as well as by failure in the supply of oxygenated blood to the centre, as may be caused by a severe operation, especially if attended by hæmorrhage[1].

The essential stimulus to inspiration which is constantly in operation is chemical and is afforded by the CO_2 tension of the blood circulating in the bulb [Haldane]. The inspiration is cut short as soon as the pulmonary alveoli become distended. The distension mechanically excites the endings of afferent fibres of the vagus; these convey impulses to the centre, reducing its excitability so that it ceases to respond to the constant stimulus. This inhibition affects both the active contraction of the muscles of

[1] The Cheyne-Stokes phenomenon with its prolonged pauses affords a striking instance of the effect of failure of the supply of blood to the centre.

inspiration and their tonus[1]. As a result of this inhibition the elastic reaction of the lungs and of the thoracic walls causes the alveoli to become diminished in size and expiration results.

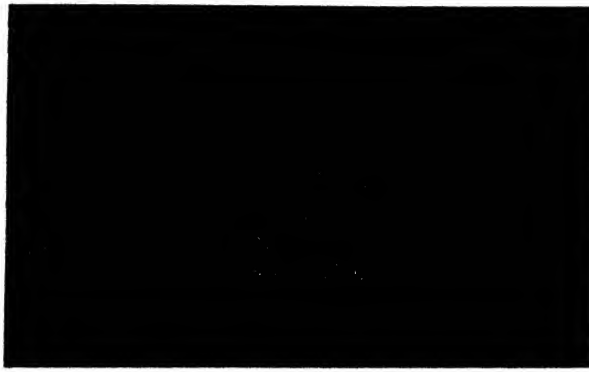

Fig. 1. From a man, aged 44. In this and the other cases from man, the respirations were recorded by Marey's pneumograph placed over the epigastrium, the pulse beats by a sphygmoscope connected with the arm-band of a Riva-Rocca.

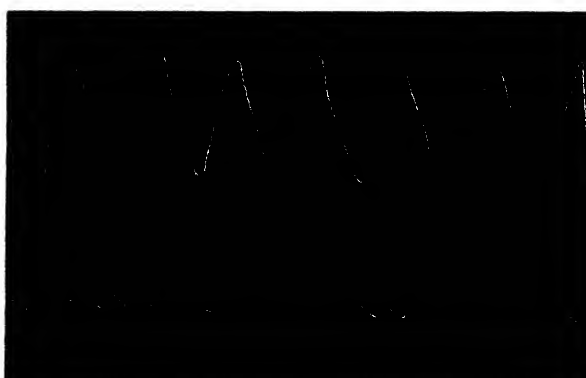

Fig. 2. From a lad, aged 16.

But expiration is not a purely passive phenomenon. It is always accompanied by some contraction of the muscles of expiration; this is better marked in quadrupeds than in man, but is never absent. What exactly is the cause of this contraction is uncertain. It was assumed by

W. R. Hess [1930], employing a mechanical method, has emphasised the effect of the condition of distension of the alveoli in modifying the tonus both of the diaphragm and of the thoracic muscles. Keller and Loeser [1930] have shown the constant presence of such tonus and have studied its variations in different conditions of inflation.

Hering and Breuer [1868] that the distension of the alveoli not only produces inhibition of inspiration but at the same time excites expiration. That afferent fibres of the pulmonary vagi can be stimulated by deflation

Fig. 3. From a man, aged 36. (In this figure the downstroke is inspiration, in all the others the upstroke.)

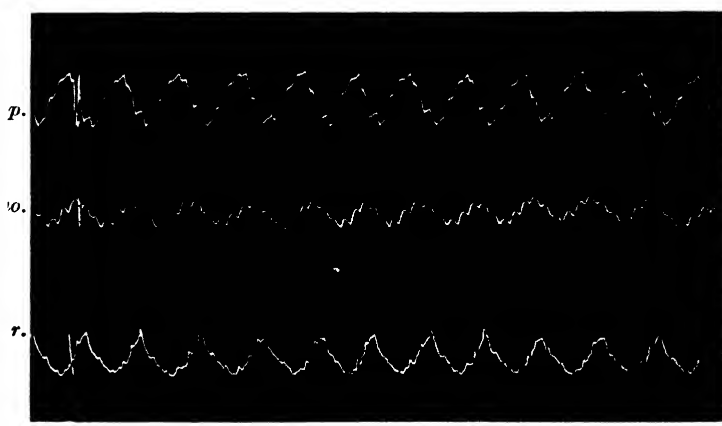

Fig. 4. From a dog, anæsthetized with chloralose. *r.* tracing of respirations; *p.* respiratory fluctuations of pulmonary blood-pressure; *ao.* respiratory fluctuations of aortic blood-pressure.

and that these act antagonistically to those excited by distension of the alveoli, increasing the excitability of the inspiratory centre to the continued stimulus of CO_2, is unquestionable, but it is still controversial whether they are called into play in quiet respiration.

According to Schenck [1903] this only occurs in forced expiration. Adrian [1926] found that oscillations of the capillary electrometer were at a minimum during expiration,

but Wachholder and McKinley [1929] noticed with every change of volume of the lungs increased oscillations of the string galvanometer, indicating an increase of strength of tonic impulses transmitted by the vagi.

The result of this alternation of excitation and inhibition constitutes the "Selbst-steuerung" of Hering and Breuer, who demonstrated it by the classic experiment of closing the trachea in the inspiratory and expiratory phases respectively.

All later investigators have confirmed their results, *e.g.* Gad [1879], Head [1889], Haldane and Mavrogordato [1916] (in man), and many more. The phenomena are strikingly exhibited by breathing into and out of a large reservoir of air maintained at a positive or a negative pressure some 20 cm. H_2O above or below the atmospheric pressure [Stefani and Sighicelli, 1888].

That there are two sets of afferent fibres in the vagi having antagonistic actions on respiration was first shown by Traube [1847] and generally recognized. Stimulating the central end of a cut vagus provokes either increased or diminished activity of respiration according to the character and intensity of the stimulation.

Since the rate and depth of respiration is usually profoundly affected by section of the vagi it is commonly assumed that respiration, so far as its rhythm is concerned, is regulated solely through those nerves. But there are reasons against this view. For the rhythmic succession of inspiration and expiration is continued after section of both vagi. True, it is generally at a slower rate, but, as Gad was the first to point out, this is not always the case. And if the obstructive dyspnœa which results from section of the laryngeal fibres is avoided, the slowing is never permanent, and, even when well marked at first, disappears after a time. As Christiansen and Haldane [1914] showed, any agency which obstructs the passage of air causes respiration to be slower and deeper. This is a constant feature of the obstruction caused by paralysis of the vocal cords—or even of one cord only. It can be avoided by cutting the vagi below the place where they give off the inferior laryngeals: if this is done no permanent slowing results [Boothby and Shamoff, 1915]. It can also be obviated by previous cauterization of the glottis [Sharpey-Schafer, 1919]. As was there shown and illustrated by numerous examples and tracings, in some animals section of both vagi may produce either no slowing, or such slowing as occurs is not permanent[1].

[1] The authors of a recent article in the *J. Physiol.* [Hammouda and Wilson, 1932] "on the function of the vagus in respiration" attribute to me the opinion that the slowing of respiration which usually immediately follows division of the vagi is caused by this dyspnœa. I have nowhere expressed such an opinion. The authors have either not read my paper carefully or have misunderstood my statements.

There must, therefore, be some regulating mechanism besides that described by Hering and Breuer. What this other mechanism is there can be no difficulty in deciding. For, as Fleisch [1928] has shown, the respiratory muscles are subject to the same conditions as to reciprocal innervation as the skeletal muscles, in which contraction reflexly provokes inhibition of their antagonists.

We may suppose that normally both vagal and muscular mechanisms are in operation, although in most cases the vagal mechanism predominates. In the absence of one mechanism the other may take on the regulating function alone. That the muscular mechanism can operate by itself was shown by A. D. Macdonald and myself (1925) in an experiment on a dog, which was brought to the notice of the Society a few years ago. In this experiment the muscular and skeletal respiratory mechanism of the thorax was reduced to a ring, formed by three ribs with their intercostal muscles and a section of the sternum. The three ribs were severed from the rest but retained their articulations with the vertebral column as well as the vascular and nervous supply to the intercostals. When the tension of CO_2 in the blood was allowed to increase by arrest of artificial respiration, the ring moved forwards and backwards with a slow steady rhythm; forwards by contraction of the external intercostals, and backwards by contraction of the internal intercostals. Since the pulmonary alveoli were passive, the regulation of the rhythm must have depended upon the alternation of excitation and inhibition of the neuromuscular mechanism.

It may be suggested that the respiratory centre itself works with a rhythm of its own, but experimental evidence of the existence of such rhythmic automatism is still lacking[1]. It used to be believed that it was established by a well-known experiment of Rosenthal, who obtained rhythmic respiratory movements of the nostrils after he had supposedly cut off all afferent impressions from the centre. But the reciprocal innervation of muscles was then unknown, and the afferent impressions emanating from the muscles involved had not been cut off.

[1] Adrian and Buytendijk [1931] have shown that the isolated brain stem of the goldfish exhibits oscillations of potential synchronous with the respiratory movements of the gills, and consider that the waves represent a slow change in the nerve cells or dendrites, since they occur in the entire absence of sensory impulses. This observation certainly furnishes an argument in favour of the rhythm of respiration having a central origin—but the argument cannot be pressed too far, for the mechanism of respiration is quite different in fishes from the far more complex conditions found in mammals.

SUMMARY.

1. Respiration consists of inspiration and expiration: normally there is no pause.

2. No definite relationship obtains between the frequency of the pulse and that of quiet respiration, but usually there are 5 to 8 heart beats to each respiration.

3. Expiration is never a passive process but is always accompanied by muscular activity.

4. Although the regular rhythm of respiration is caused by the inflation and deflation of the pulmonary alveoli acting through the vagi it is assisted by afferent impulses emanating from the respiratory muscles. If the vagi are severed these impulses may be sufficient to maintain a normal rhythm.

5. It is doubtful if the respiratory centre has a rhythm of its own, independent of all reflexes. In the experiments which have been thought to prove its automatism the afferent impulses from the respiratory muscles have not been taken into account.

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CREATINE AND PHOSPHORUS COMPOUNDS IN MALIGNANT TUMOURS.

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EXCEPT in a few isolated cases, creatine has been found solely in vertebrate animals. The ordinary forms of malignant tumour also seem to be limited to vertebrates, and so it appeared conceivable that the occurrence of creatine could have some relation to malignant disease. Edlbacher and Kutscher [1931], however, found no creatine phosphate nor arginine phosphate in tumours. The type of tumour used in their experiments is not stated and no units are given for any of their results. Since then Franks [1932] has shown that the Crocker Institute mouse tumour 180 (a sarcoma) contains a small amount of phosphagen as estimated by the method of Eggleton and Eggleton [1929]. The experiments described below, which were completed before the appearance of Franks' work, show that some phosphagen occurs in tumours. The phosphagen content of tumours is indeed only one-twentieth that of normal resting voluntary muscle, while the creatine content is about a tenth of the amount found in such muscle. The low phosphagen content, however, does not necessarily mean that this substance is of no significance in tumour metabolism. Clark, Eggleton and Eggleton [1931] have shown that normal hearts contain only a tenth as much phosphagen as skeletal muscle, yet these authors [1932] have shown that the amount of phosphagen, small though it is, is essential for cardiac metabolism.

Perhaps the most striking feature in the distribution of the phosphorus compounds in tumours is the large amount of total phosphorus present. Although there is less acid-soluble phosphorus present than in muscle, the amount of nucleic acid and nucleoprotein is three or four times as great. It would appear that malignant tissue would form as good a starting material as thymus gland for the preparation of nucleic acid.

Edlbacher and Kutscher [1931] found an active nucleotide phosphatase in tumours, and this is confirmed by Franks' [1932] findings that on incubation the total acid-soluble phosphorus of tumour tissue in-

creased. In the latter experiments some of the increase in acid-soluble phosphorus would be accounted for by autolysis of nucleic acid. Experiments described below show that on incubation in bicarbonate solution without carbohydrate there is no phosphate esterification, but a considerable production of free orthophosphate. Edlbacher and Kutscher also found this production of free phosphate, and state further that this process continued even in the presence of fluoride and carbohydrate. Franks, however, found some esterification when glucose alone was added. The glycolysis of tumours in the presence of glucose is much greater than the esterification. In an hour, twenty to sixty molecules of lactic acid are formed for each atom of phosphorus esterified, but even this esterification is stopped by the presence of fluoride [Edlbacher and Kutscher, 1931], which has been shown to inhibit glycolysis of malignant tissue [Dickens and Šimer, 1929]. Edlbacher and Kutscher, and Franks, found pyrophosphate in tumours. Edlbacher and Kutscher apparently found as much pyrophosphate as free inorganic phosphate. Franks' highest figure for pyrophosphate is equal to about half the amount of orthophosphate and that is the order of the amount found in the present investigation. From the results given below it is obvious that the increase in free phosphate on incubation is largely at the expense of this pyrophosphate fraction, and this would appear to be the case in the experiments of Edlbacher and Kutscher, and of Franks (though in neither paper is this stated). The part of the phosphorus metabolism of incubated tumour tissue which can be observed is therefore mainly a hydrolysis of adenylpyrophosphoric acid to give free orthophosphate and inosinic or adenylic acid. At the same time there is an increase in acid-soluble phosphorus due to hydrolysis of nucleic acid. Even in the presence of carbohydrate when rapid glycolysis occurs the free orthophosphate tends to increase. This fact could be accounted for either by the extraordinary activity of the phosphatases in the system, or by the fact that the free orthophosphate in the trichloroacetic acid extract is not present as such in the tissue, but is bound in some labile compound in which condition it is incapable of undergoing esterification.

Estimation of creatine.

Creatine and creatinine were estimated colorimetrically by Folin's [1915] method in which the colour given with alkaline picrate is compared with the colour given by a standard creatinine solution. Tumours immediately after removal were divided into two parts, one of which was

ground with sand and picric acid and used for the creatinine estimation, while the other was heated at 125° C. for half an hour with N H_2SO_4 to convert creatine into creatinine. After the first estimations it was found that the creatinine content (1·5, 1·8, 2·5, 1·2 mg. per 100 g. tissue of Jensen rat sarcoma) was as small as that in blood or muscle and could be neglected, and that hydrolysis of the whole tissue with sulphuric acid gave brown solutions which reduced the accuracy of the creatine estimation. In most of the experiments, therefore, creatine was estimated on the trichloroacetic acid extract of the tissue which was prepared as for the estimation of phosphorus compounds. The first results (Table I) were obtained from human tumours, which were generally analysed within 1 or 2 hours after their removal from the subject.

TABLE I. Creatine content of human tumours.

				Normal tissue of same person		
Case	Type of tumour	Weight of tissue (g.)	Creatine (mg. per 100 g.)	Type	Weight of tissue (g.)	Creatine (mg. per 100 g.)
a	Carcinoma of breast	20·5	11	Breast	27·1	5·2
	Metastases in glands	2·78	104	Pectoral muscle	9·7	390
b	Carcinoma of breast	19·6	50·5	Breast	16·1	Under 5
c	Carcinoma of rectum	21·4	64	Rectum (mucous membrane and muscular coats)	11 9	121
d	Carcinoma of breast	15·7	14	—	—	—
e	Carcinoma of rectum	17·4	11·5	Mucous membrane of rectum	11·5	15

It will be seen that there is great variation in the creatine content of the tumours examined. This is probably due to the fact that such samples may be contaminated with surrounding tissue, which masks the true content in different types of tumours. They serve at any rate to show that human tumours contain creatine. The highest figure (104 mg. per 100 g.) is given by the glandular metastases from a breast cancer; in these there would be very little admixture with other tissues.

In Table II is shown the creatine content of some tumours of the rat and mouse, namely (*a*) a primary spindle-celled tumour of the rat produced experimentally by subcutaneous injections of 1:2:5:6-dibenzanthracene in a fatty medium [L.R. 66: Burrows, Hieger and Kennaway, 1932]; (*b*) grafted tumours of the rat derived from another primary spindle-celled tumour produced in the same way as (*a*); these grafted tumours, which are denoted throughout this paper by the reference number L.R. 10, were obtained from the ninth and twelfth

transplanted generation; (*c*) the Jensen rat sarcoma; and (*d*) spontaneous mammary carcinomas of the mouse.

The primary tumour L.R. 66, and the tumours of the same type in the ninth and twelfth grafted generations (L.R. 10, Tables II and IV) contain amounts of creatine of the same order. Two of the tumours examined contained more creatine than has been found in numerous other estimates which have been made on similar tumours. Some determinations were made on the necrotic tissue of large tumours, and in these the creatine content was lower than in whole tumours or in their growing edge, showing that the creatine must be contained largely in the living tissue.

TABLE II. Creatine content of rat and mouse tumours.

Type of tumour	Tumour (g.)	Creatine (mg. per 100 g.)	Normal skeletal muscle from same animal: Muscle (g.)	Normal skeletal muscle from same animal: Creatine (mg. per (100 g.)
Rat				
Grafted L.R.10 tumour	5·0	59	3·0	536
,, ,,	5·0	57	—	—
Jensen sarcoma	30·0	105	5·0	560
,,	18·35	90	5·0	580
			5·0	630*
			5·0	590*
,,	6·0	45	—	—
,,	7·2	41	—	—
Primary 1:2:5:6-dibenzanthracene-lard tumour. L.R. 66	10·0	31	—	—
Jensen sarcoma, necrotic material	8·0	14	—	—
,, growing tissue	48	46	—	—
,, necrotic material	4·8	11	—	—
,, two whole tumours	54	27	—	—
Mouse				
Spontaneous mammary carcinoma	6·6	36	—	—
Spontaneous mammary carcinoma (two tumours pooled)	4·8	44	—	—

* Normal rat muscle.

Most of the animal tumours examined contained 30–60 mg. creatine per 100 g., and this amount is greater than that occurring in any normal tissue except skeletal muscle, heart, nerve and testis, which contain about 500, 250, 100 and 100 mg. per 100 g. respectively. It is less than the free creatine of muscle (*ca.* 95 mg. in 100 g.) as found by Walpole's method, but it is much larger than the blood creatine (*ca.* 5 mg. per 100 g.), and may therefore be of significance. Further experiments are to be made on this subject.

Phosphorus compounds in tumours.

Rats weighing 150–250 g., with large grafted tumours of connective tissue (10–40 g.), were killed by a blow on the head and the tumour rapidly removed, weighed and well ground in 10 p.c. trichloroacetic acid containing ice. After filtration the protein precipitate was well pressed on the filter and the filtrate immediately used for estimation of the phosphorus fractions. Phosphorus was estimated colorimetrically after Martland and Robison [1926]. In a preliminary orientating experiment in this way seven tumours (Jensen rat sarcoma) weighing together 104 g. were extracted with 250 g. of ice and trichloroacetic acid. Various phosphorus fractions were estimated in the acid extract and in the precipitate from which nucleic acid was extracted by warming with dilute caustic soda. The results obtained are given in Table III.

Table III. Phosphorus compounds in rat tumours and frog muscle, expressed as mg. P per 100 g. tissue.

	L.R.10 tumour	Jensen rat sarcoma	Frog muscle
Total phosphorus	254	235	182
Total acid-soluble phosphorus	52	56	134
Free orthophosphate	25	22	15*
Phosphagen (Meyerhof and Lohmann, 1928, precipitation method)	2·5	—	—
Phosphagen (Eggleton and Eggleton, 1929, extrapolation method)	1·7	1·2	65*
Pyrophosphate (P hydrolysed in N HCl at 100° in 7 min.)	11·5	12·2	25*
Hexose diphosphate (calculated from P hydrolysed in N HCl at 100° in 30 min.)	0·5	—	Trace*
Hexose monophosphate (P sol. as barium salt)	8·0	—	8*
Other acid-soluble P (nucleic acid and adenylic acid)	7·0	—	30*
Total acid-insoluble phosphorus	202	179	48
Total acid-insoluble phosphorus soluble in NaOH (nucleic acid and nucleoprotein)	196	152	43

* From Eggleton [1929].

The amount of phosphorus of tumours which is not soluble in acid is remarkably high. It would seem that tumour tissue contains three or four times as much nucleic acid as a normal tissue, such as muscle. The difference in nucleic acid content between tumour and other tissue is enormously greater and probably more significant than the difference in lecithin content found by Jowett [1931].

As stated in the introduction the free orthophosphate content is sometimes high, being greater than that present in resting muscle. In Franks' experiments incubation with carbohydrate did not remove this free phosphate, and it is possible that it is actually in some labile combination

which is rapidly hydrolysed even when removal and treatment with trichloroacetic acid containing excess of ice are carried out rapidly. Experiments were made in which the tissue was frozen with carbon dioxide snow in some cases and liquid air in others before extracting with trichloroacetic acid, but even under such conditions the free orthophosphate was often more than 20 mg. per 100 g. tissue. The extract made from frozen tissue was always very cloudy, so that the results were not so accurate as those obtained by the usual procedure.

Next to the free orthophosphate, the pyrophosphate is the most plentiful of the acid-soluble fractions. Lohmann [1931] has shown that pyrophosphate is really combined with adenylic acid in the form of adenyltriphosphoric acid in skeletal muscle (with 2 atoms of labile P to each atom of "organic" P); and in the form of adenyldiphosphoric acid (with 1 atom of labile P to each atom of "organic" P) in heart muscle. In the presence of magnesium salts these compounds act as co-enzyme in lactic acid production. It was of interest therefore to isolate and examine the pyrophosphate fraction of tumours. This was done by Lohmann's method, precipitating as barium salt, then as mercury salt and finally removing the inorganic phosphate by precipitation with ammoniacal magnesia mixture. The resulting compound precipitated as barium salt contained 1·9 atoms of labile P to each of organic P in one preparation from grafted L.R.10 tumour, and 2·08 atoms of labile P to one atom of organic P in a sample prepared from Jensen rat sarcoma. The co-enzyme or adenylpyrophosphoric acid of malignant tissue would therefore appear to be the same as that obtained from skeletal muscle.

The effect of survival on the acid-soluble phosphorus compounds including phosphagen was next determined. Large grafted rat tumours were rapidly excised and cut in half. One half was immediately ground with ice and trichloroacetic acid while the other half was first allowed to stand in Ringer-bicarbonate for 1 or 2 hours. Phosphagen was estimated by the extrapolation method of Eggleton and Eggleton [1927] and by Lohmann's method [1928] in which orthophosphate is precipitated with ammoniacal magnesium citrate[1]. There are some discrepancies in the results obtained for phosphagen as estimated by the precipitation and extrapolation methods. This may be due to a small amount of arginine phosphate which is not estimated by the extrapolation method. On the other hand the extrapolation method is much less accurate when applied

[1] Estimations made by the method of barium precipitation after Eggleton and Eggleton [1929] gave results comparable with those obtained by Meyerhof's method, *e.g.* four consecutive experiments gave 2·7, 2·6, 4·1 and 3·5 phosphagen P per 100 g.

to small amounts. According to both methods of estimation there is an autolysis of phosphagen on incubation. Typical results are shown in Table IV.

TABLE IV. Creatine and acid-soluble phosphorus compounds in grafted rat tumours expressed as mg. P per 100 g. moist tissue.

Rat No.	Type	Condition	Creatine content	Free ortho-phosphate	Phosphagen P estimated by Pre-cipitation	Phosphagen P estimated by Eggleton extra-polation	Pyro phos-phate	P after ½ hour hydrolysis in N HCl (hexose di-phosphate)	Total acid-soluble phos-phorus
1	J.R.S.	Freshly excised	29	20·0	—	1·8	—	—	50·8
2	J.R.S.	Stood 1 hour in Ringer	27	27·2	—	0·2	—	—	52·9
3	J.R.S.	Stood 2 hours in Ringer	33	32·4	—	0·7	—	—	55·0
4*a*	L.R. 10	Freshly excised	31	16·5	3·2	0·6	12·3	0·5	52·0
4*b*	,,	Stood 1 hour in Ringer	33	22·0	2·4	0·7	8·9	0·7	55·4
5*a*	,,	Freshly excised	31	24·4	3·9	1·0	11·2	1·1	56·4
5*b*	,,	Stood 2 hours in Ringer	34	34·7	0·7	Nil	4·0	0·6	60·2
6*a*	,,	Freshly excised	30	18·5	2·3	2·4	16·2	0·8	54·3
6*b*	,,	Stood 1 hour in Ringer	31	26·8	1·1	0·4	11·1	0·8	59·0

It can be seen that survival leads to a rise in orthophosphate content and a smaller rise in the total acid-soluble phosphorus. It is evident that this free orthophosphate is derived partly from phosphagen and the acid-insoluble fraction. This latter accounts for the increase in total acid-soluble phosphorus. The effect must be partly due to the active nuclease found by Edlbacher and Kutscher, but it must also be in part due to the high concentration of substrate present upon which such an enzyme can act. Ashford and Holmes [1931] have recently found a similar increase of acid-soluble phosphorus on incubation in brain tissue. The largest part of increase in orthophosphate is, however, due to hydrolysis of adenylpyrophosphate; the occurrence of this hydrolysis shows that tumours contain pyrophosphatase. These results explain those of Franks, in which under similar conditions free phosphate was formed. In the presence of glucose this free phosphate would be esterified to hexose monophosphate which, as Robison and Morgan [1930] showed, is precipitated to a considerable extent by 10 p.c. alcohol when inorganic phosphate is present. Franks found in muscle a change in the opposite direction (*i.e.* a decrease in the phosphorus precipitated as barium salt by 10 p.c. alcohol), and this appears to be due to decomposition of creatine phosphate. The important change in the phosphorus

compounds of tumours on incubation is therefore due to pyrophosphatase hydrolysing adenylpyrophosphoric acid, which is the co-enzyme of lactic acid formation.

SUMMARY.

1. Human tumours contain creatine. The wide range in the amount found is probably due to varying admixture with other tissues.

2. The majority of the tumours from rat and mouse examined contained very little creatinine but 30–60 mg. creatine per 100 g. tissue. In freshly excised tumour, and hence probably *in situ*, a quarter to a third of the creatine is combined with phosphate as judged by phosphagen estimations. The phosphagen breaks down on standing.

3. The amount of acid-insoluble phosphorus in malignant tumours is very high (200 mg. per 100 g.), being about four times as great as in skeletal muscle.

4. Tumours contain practically as much adenylpyrophosphoric acid as does skeletal muscle and the compound would appear to be the same as in skeletal muscle. This compound is hydrolysed on incubation of the tumour; hence a pyrophosphatase must be present.

In conclusion I wish to thank Prof. E. L. Kennaway for help and advice on various matters.

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ON VASO-DILATOR FIBRES IN THE SYMPATHETIC, AND ON THE EFFECT OF CIRCULATING ADRENALINE IN AUGMENTING THE VASCULAR RESPONSE TO SYMPATHETIC STIMULATION.

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Part I.

The evidence to be presented in this paper may be conveniently divided into two parts of which the first deals with the existence of vaso-dilator fibres in the sympathetic system. At the present time opinion on the existence of these fibres differs in different schools; while some do not believe in them, others are already convinced of their existence. It is generally accepted that, locally, vaso-dilator fibres exist; the work of Dastre and Morat [1880] demonstrated their presence in the branches of the cervical sympathetic leading to the bucco-facial region. The strongest evidence for a general distribution of vaso-dilator fibres was that obtained by Dale [1906] who showed that in the cat, after the administration of ergotoxine, injection of adrenaline and splanchnic stimulation produced vaso-dilatation; since ergotoxine paralysed all sympathetic effects which were purely motor, and left unaffected those which were inhibitor, this reversal of the normal vascular response pointed to the existence of a general system of vaso-dilator fibres. Recently Lewis and Pickering [1931] have obtained evidence of the existence of vaso-dilator fibres in the sympathetic in human subjects. They place a man in a cold room until his hands are cold, and then in a warm chamber with his hands outside; after an interval the skin temperature of the hands rises as a result of the rise of body temperature. The efferent path of the impulses which lead to rise of the temperature of the hands is the sympathetic, for in a subject in which one arm has been deprived of its sympathetic nerves, the reflex rise of temperature occurs only in the hand of the normal arm. The rise of temperature in a healthy subject might be explained by loss of vaso-constrictor tone, since a

similar rise of temperature in the appropriate area follows anæsthetization of the ulnar nerve at the elbow. This explanation, however, does not account for the fact that patients with Raynaud's disease behave differently; in them the warm chamber will cause a rise of hand temperature, but anæsthetizing the ulnar nerve will not. Lewis and Pickering conclude that, since, in these patients, anæsthetizing the ulnar nerve does not warm the hand, the effect of the warm chamber must be exerted by dilator impulses rather than by loss of vaso-constrictor tone. In support of this they show that when a patient with Raynaud's disease is put in the warm chamber with the ulnar nerve anæsthetized, then reflex warming of the hands does not occur in the ulnar area, although it occurs elsewhere.

The experiments described in this paper are the unexpected outcome of work begun some two and a half years ago on the cardio-vascular action of tyramine. The peculiar fact was observed [Burn, 1930] that when the hindlimbs of the cat or dog are perfused with defibrinated blood, the intra-arterial injection of tyramine is attended by only a trace of constrictor effect, but that if adrenaline is added to the blood so as to produce a steady concentration, and to maintain a raised peripheral resistance in the perfused vessels, then the effect of the intra-arterial injection of tyramine is greatly enhanced. It was next found [Burn and Tainter, 1931] that tyramine, and also ephedrine, differ from adrenaline in their action on the denervated pupil of the cat's eye, inasmuch as they have little or no dilator effect when the sympathetic nerve fibres have degenerated, whereas adrenaline acts more powerfully than in the normal eye. The same phenomenon was then observed [Burn, 1932] in the vessels of the cat's forelimb; after degeneration of the sympathetic fibres consequent on the extirpation of the stellate ganglion, tyramine and ephedrine lose their constrictor action, while that of adrenaline remains. In the same paper evidence is given that the peculiar action of adrenaline in enhancing the constrictor effect of tyramine in perfused vessels is not exerted by pituitary extract, and also that the constrictor action of ephedrine, like that of tyramine, is small in the absence of circulating adrenaline and greatly increased by its presence.

It seemed that there might be a link between the two phenomena; perhaps a substance which acted on the sympathetic nerve ending itself, and which failed to act when the ending had degenerated, required a certain concentration of circulating adrenaline because of its site of action. Perhaps the effect of circulating adrenaline on the action of tyramine and ephedrine might be due to the effect of the adrenaline on

the sympathetic nerve endings; if this were so it was important to investigate the influence of circulating adrenaline upon the magnitude of the response to sympathetic stimulation. This investigation was undertaken and its results are now to be described.

Experimental methods.

The experiments have been performed by perfusing the hind part of dogs through the abdominal aorta after removal of the viscera.

One dog was used to obtain a supply of blood, and the second for the perfusion. The first dog was anæsthetized with chloroform and ether, and a cannula was then inserted in the carotid artery. Blood was collected in a glass vessel and defibrinated. The blood when filtered through muslin was kept in a bath at 37° C. The second dog was similarly anæsthetized, and a cannula was inserted in its trachea. A cannula was inserted in the external jugular vein, and chloralose administered in a dose of 0·1 g. per kg. body weight; the volatile anæsthetic was then discontinued. The abdomen was then opened in the middle line, and the rectum divided between double ligatures. The inferior mesenteric artery was divided between ligatures. Since complete evisceration is difficult in most dogs without opening the chest, the chest was opened by a careful mid-line incision and artificial respiration applied. Small bleeding points were cauterized with a hot iron. The superior mesenteric artery and the cœliac axis were divided between ligatures. The cardiac end of the stomach was divided between ligatures, the portal canal divided between ligatures, and the stomach, intestines, pancreas and spleen then removed. The blood vessels in the mesentery lining the posterior abdominal wall were then divided so as to clear the area on the left side of the abdominal aorta; both kidneys were removed and the left suprarenal gland was excised. A loose ligature was then passed round the inferior vena cava just below the entrance of the left suprarenal vein. A series of five stout mass ligatures to enclose the whole cross-sectional area of the body wall were placed in position as close to the lowest rib as possible. The dog was then killed by allowing its blood to run out through a cannula in the carotid artery. The dog was divided into two parts at the lower end of the thorax, the spinal column being sawn through above the diaphragm. Sufficient of the upper ribs were cut away with bone forceps to allow a cannula to be inserted into the pulmonary artery and a second into the tip of the left auricle. A stout string was tied around the ventricle near the base. The lungs were then perfused with defibrinated blood driven by one chamber of a Dale-Schuster [1928] pump, and were ventilated

through the trachea with a mixture of oxygen and 5 p.c. carbon dioxide. This mixture was blown through water at 37° C.

The pulmonary circulation having begun, the lower half of the dog was prepared for perfusion. The open end of the spinal canal was plugged with plasticine. The diaphragm and the liver were cut away. The abdominal aorta was dissected with care and ligatures passed around it for the insertion of a cannula at the level of the left renal artery. The left sympathetic chain was exposed and cleared from other structures. The mass ligatures round the body wall were tied, care being taken not to include the aorta or the sympathetic chains on each side. The aortic cannula was then inserted and also a cannula in the inferior vena cava just below the entrance of the right suprarenal vein. The right suprarenal gland was cut away. The aortic cannula had a bubble trap attached, and a side-piece which was connected to a mercury manometer; the connecting tube was filled with Ringer's solution. Perfusion through the aortic cannula was then begun by means of the second chamber of the Dale-Schuster pump. The first 300 c.c. of blood issuing from the vein were collected, whipped and filtered before being returned to the system. The blood in the system was now pumped alternately through the lungs and through the hind part of the dog. The tissues of the dog very powerfully reduced the blood, and the lungs re-oxidized it so that the colour was once more bright red. Occasionally the lungs did not act efficiently; such an experiment was disregarded.

The left (sometimes the right) sympathetic chain was then prepared for stimulation at a point about half-way between the renal artery and the aortic bifurcation opposite the fourth and fifth lumbar vertebræ. In earlier experiments the chain was divided and the lower end was stimulated; in later experiments two platinum electrodes were hooked under the chain and this was raised out of contact with other structures though not stretched. The electrodes were held rigidly in a clamp, and the portion of chain to be stimulated was surrounded by cotton-wool moist with Ringer; before stimulation the portion of chain between the electrodes was dried by touching it with an edge of filter paper. The stimuli were induction shocks from a primary circuit containing a 2-volt accumulator and a Lewis's rotary contact breaker adjusted to give 18 breaks shock per second in the secondary circuit; when an ordinary magnetic interrupter is used in the primary, it is difficult to ensure that the rate of interruption is always the same. The preparation of the system described took from 3 to 4 hours.

Experimental observations.

When the perfusion through the lungs and through the hind part of the dog has begun, the height of the pressure in the aortic cannula depends upon the output per stroke of the pump, the rate of the pump and the resistance of the vessels in the hind part of the dog. In the experiments to be described the output per stroke and the rate were maintained constant throughout each experiment, the output per minute being about 80 to 100 c.c. and the rate being 92 per min. The pressure recorded in the aortic cannula was low in these circumstances, being only 20–30 mm. Hg. To the reservoir receiving the blood from the inferior cava, a slightly acidified dilution of adrenaline (1 in 20,000) was added at a constant rate from a burette. The rate varied from 0·3 to 1·0 c.c. per min. in different experiments. The blood containing this adrenaline was pumped in succession through the lungs and then through the hind part of the dog, in which it produced a rise in the peripheral resistance. The pressure in the aortic cannula rose, and it was possible to adjust this pressure to any given height by controlling the amount of adrenaline added from the burette; naturally any change in the rate of adding adrenaline was only followed by a change in the height of the pressure in the cannula after a few minutes' delay.

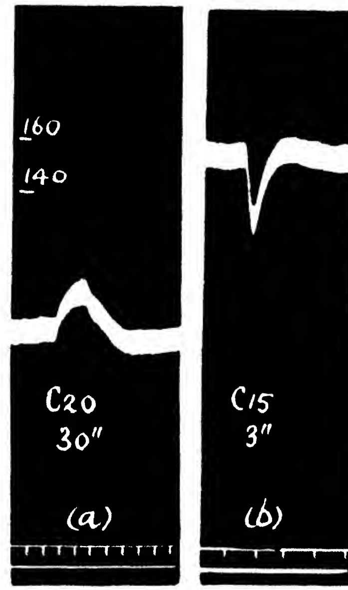

Fig. 1. Record of pressure in cannula in abdominal aorta of dog, the hind part of which was perfused with blood. *a* shows constrictor effect of stimulating the lumbar sympathetic chain for 30 sec. with secondary coil at 20 cm. *b* shows dilator effect of similar stimulation for 3 sec. with coil at 15 cm.

When the peripheral resistance had risen so that the cannula pressure reached 100–130 mm. Hg., and had been maintained there for 30–45 min., it was found that stimulation of the sympathetic chain was not always attended by vaso-constriction and a consequent rise in the cannula pressure. In Fig. 1 is shown the vaso-constriction produced by stimulating the sympathetic chain for 30 sec. with the secondary coil at 20, and also a vaso-dilatation produced by stimulation for 3 sec. with the secondary coil at 15. The vaso-constriction was produced when the

cannula pressure was about 70 mm. Hg. and the vaso-dilatation was produced when the cannula pressure was 150 mm. Hg.

In the circumstances of these experiments it was not found that vaso-dilatation was an exceptional occurrence, and that vaso-constriction was the rule; actually the observations were the opposite of this, for vaso-dilatation was easier to elicit than vaso-constriction when the sympathetic chain was stimulated. There were experiments, indeed, through-

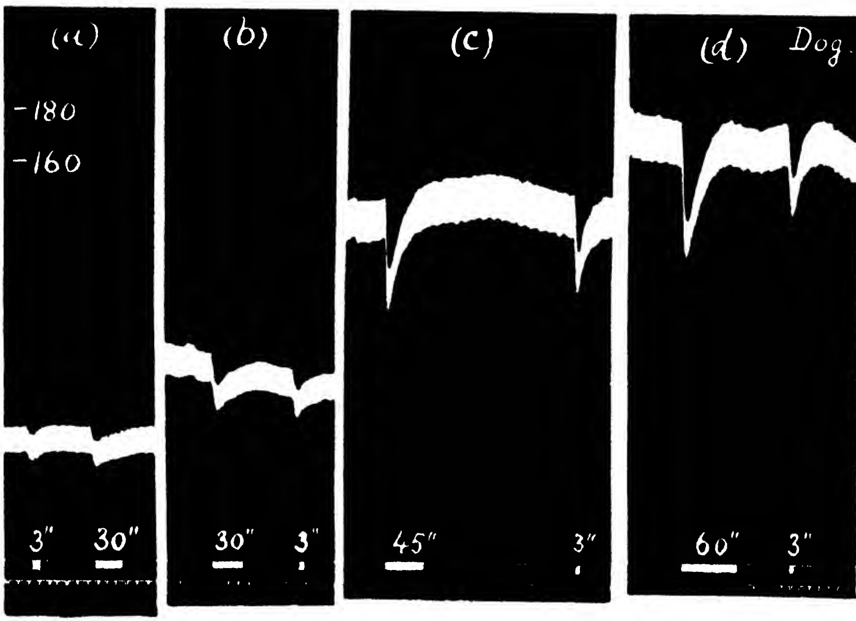

Fig. 2. Similar record to Fig. 1 in another dog. The effects of the sympathetic stimulation were all dilator, and no constriction was observed. *a* shows the responses after 0·1 mg. adrenaline was added to the circulating blood; *b* after 0·2 mg.; *c* after 0·3 mg.; *d* after 0·42 mg. Stimulation was for the duration indicated with coil at 12 cm. throughout.

out the whole of which vaso-dilatation was the only response; an example of this is given in Fig. 2. Fig. 2*a* shows the response to sympathetic stimulation when only 2 c.c. of adrenaline (= 0·1 mg.) had been added to a total blood volume of 600 c.c.; the remaining portions of the figure show the increasing vaso-dilator response when more adrenaline was added, *b* after the addition of a total of 0·2 mg., *c* after 0·3 mg., and *d* after 0·42 mg. Throughout this experiment the strength of stimulation was unchanged (coil = 12 cm.), the duration of the applied stimulus being as shown in the figure.

Some experiments fortunately permitted a closer analysis of the conditions in which sympathetic stimulation resulted in vaso-constriction on the one hand, and in vaso-dilatation on the other. It was found that at any strength of stimulus, a brief stimulus gave vaso-dilatation, while a prolonged stimulus favoured the appearance of vaso-constriction. In Fig. 3, with coil at 20 cm., stimulation for 30 sec. produced vaso-constriction, but the same stimulus applied for 3 sec. gave vaso-dilatation.

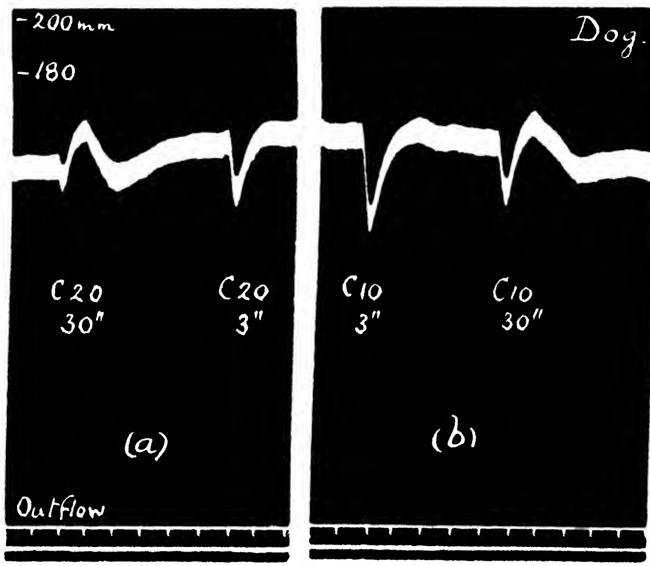

Fig. 3. Similar to Figs. 1 and 2. In *a* a constrictor response follows stimulation for 30 sec. and a dilator response follows stimulation for 3 sec., the same strength of stimulus being applied each time. In *b* a stronger stimulus shows the same difference according to the time of application. Note that stimulation for 30 sec. evokes dilatation followed by constriction and that as the constriction passes off dilatation is again evident.

The fall in the peripheral resistance when a brief stimulus was applied was seen to continue for a short time after the stimulus was stopped, and similarly the initial effect of the longer stimulus was seen to be vaso-dilatation which was quickly masked by vaso-constriction. When a stronger stimulus was applied (coil at 10 cm.) the same difference was observed between the short and the long application; the 3 sec. stimulus produced a simple though greater dilatation, and the 30 sec. stimulus resulted in a clear mixture of dilatation and constriction. It is worth careful note, that, after this longer stimulus at coil 10, the sequence was dilatation, constriction and again dilatation. The same sequence was

detectable with the longer stimulus when the coil was at 20. The significance of this point will be discussed later.

It follows from these observations that the sympathetic chain in the dog contains vaso-dilator as well as constrictor fibres; the existence of vaso-dilator fibres in the sympathetic is, therefore, placed beyond further doubt.

PART II.

The second part of the evidence presented in this paper deals with the effect of circulating adrenaline on the magnitude of the response to sympathetic stimulation; as has been explained, the work was primarily undertaken to investigate this effect. When the perfusion of the hind part of the dog is begun in the manner described, no adrenaline having been added to the reservoir collecting the venous outflow, stimulation of the sympathetic chain has very little effect on the tone of the vessels even when powerful stimuli are applied. With the secondary coil as close as 10 cm., some slight change may be observed, just big enough to be recognized as an indisputable response; weaker stimuli than this are as a rule entirely ineffective, and stronger stimuli do not evoke greater responses. In all the experiments performed, there has been no exception to this finding, which is as uniform as the previous observations that in the same circumstances tyramine and ephedrine have very little constrictor effect, in spite of the fact that the preparation is extremely sensitive to adrenaline [Burn, 1930 and 1932]. The response to sympathetic stimulation in these circumstances may be either a slight vaso-dilatation (Fig. 4) or a slight vaso-constriction (Fig. 5).

Effect of pituitary extract.

The response to stimulation is not increased by adding pituitary (posterior lobe) extract to the venous blood so as to raise the tone of the vessels and the pressure in the aortic cannula. Fig. 4*a* and Fig. 6*a* both show that the effect of sympathetic stimulation is only a slight vaso-dilatation although pituitary extract had been added to the circulating blood for 45 min. previously. In Fig. 6*b* the response remained unchanged 1 hour later after further addition of pituitary extract (a total of 30 units to 600 c.c. blood), and although the perfusion pressure was as high as 100 mm.

Effect of adrenaline.

In complete contrast to pituitary extract stands adrenaline. If adrenaline is added to the circulating blood, sympathetic stimulation whether resulting in dilatation or constriction soon becomes much more

effective; greater responses follow the application of the same or much weaker stimuli. The effect of adrenaline in increasing the vaso-dilator impulses may first be illustrated. In Fig. 4 is shown the dilator response to stimulation of the sympathetic chain for 5 sec. and 30 sec. with the secondary coil at 15 cm. In Fig. 4*a* the stimulation was applied when pituitary extract had been added to the circulating blood so that the

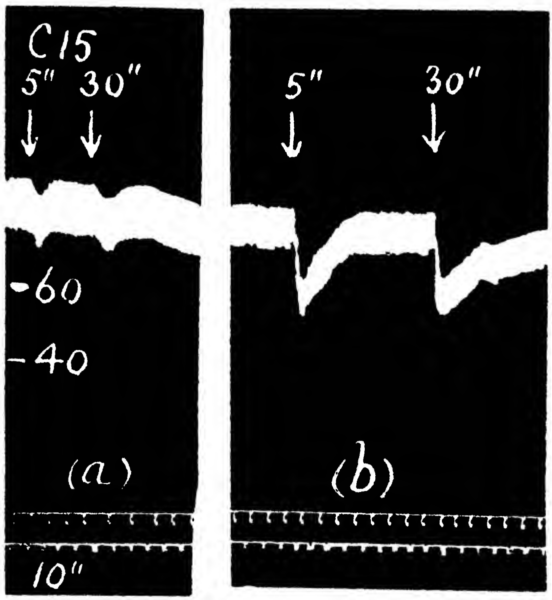

Fig. 4. The record shows in *a* the feeble dilator responses to sympathetic stimulation when pituitary (posterior lobe) extract was added to the circulating blood to raise the vascular tone. In *b* the same stimuli were applied when adrenaline was added to the blood, and greater dilator responses were obtained although the vascular tone was no higher than in *a*.

mean pressure in the aortic cannula was 80 mm. Pituitary extract had been added to the venous reservoir in amounts of 3 units every 5 min. up to a total of 30 units. The effect of stimulation having been recorded as shown, about 300 c.c. of the blood in the system was replaced by an equal volume of blood containing no pituitary extract. This newly added blood was part of the same original stock as that already perfused. When the change had been made, the tone in the vessels fell, and adrenaline (1 in 20,000) was then added steadily (0·5 c.c. per min.) to the venous reservoir. The pressure in the aortic cannula again rose; when it had

almost reached the previous level, though not quite, the addition of adrenaline was stopped, and in a few minutes the stimulation of the sympathetic chain was repeated using the same strength and duration of stimulus. Fig. 4*b* shows that the vaso-dilator response was much greater than before. It is evident that the increase was not due to the effect of adrenaline in creating vascular tone, for this was actually higher when the perfusing blood contained pituitary extract.

Not only does adrenaline augment the vaso-dilator response to sympathetic stimulation, but it augments the vaso-constrictor response also. While in some preparations the only effect of sympathetic stimulation was to produce vaso-dilatation, in some others the only effect was to produce vaso-constriction; the effect of adrenaline in augmenting the vaso-constrictor response is best seen in one of the latter. An illustration appears in Fig. 5. At the beginning of the experiment a perceptible effect was not produced in the vessels by stimulation when the secondary coil was further away from the primary than 10–12 cm. Adrenaline was then added to the venous reservoir at a steady rate, and when the cannula pressure had risen to about 130 mm., stimulation with the secondary coil at 15 or 20 cm. produced more vaso-constriction, although the vessels were already in much greater tone. The addition of adrenaline to the reservoir was stopped, and after an interval the tone in the vessels fell. In Fig. 5*d*, stimulation for 5 sec. at coil 20 was ineffective, stimulation for 30 sec. was almost ineffective, but stimulation at coil 15 provoked vaso-constriction. With further addition of adrenaline, stimulation at coil 20 became once more effective, and as shown in Fig. 5*g* and *h*, remained effective after the adrenaline was again discontinued, and the tone allowed to disappear completely. The record shows that the addition of adrenaline not only raised the vascular tone, but augmented the response to sympathetic stimulation, and that the two effects were not connected. For comparison of Fig. 5*a*, *d*, *g* and *h*, in all of which the effect of adrenaline on vascular tone was slight, shows a steady increase in the augmentation of the response to sympathetic stimulation the longer adrenaline has been present in the blood. It is true that as the tone becomes less, the efficiency of the stimulation becomes less (cf. Fig. 5*g* and *h*), but the two effects do not increase and diminish in parallel; the augmentation of response to stimulation persists much longer. The greatest responses were certainly obtained when the tone in the vessels was greatest, but no suggestion has yet been made in physiological work that the more vessels are constricted, the more easily can constrictor effects be elicited in them; hence even the records in Fig. 5*b*, *c*, *e* and *f* are

good evidence that adrenaline augments the response to stimulation apart from its effect on vascular tone; the effect is evidently exerted in spite of its effect on vascular tone.

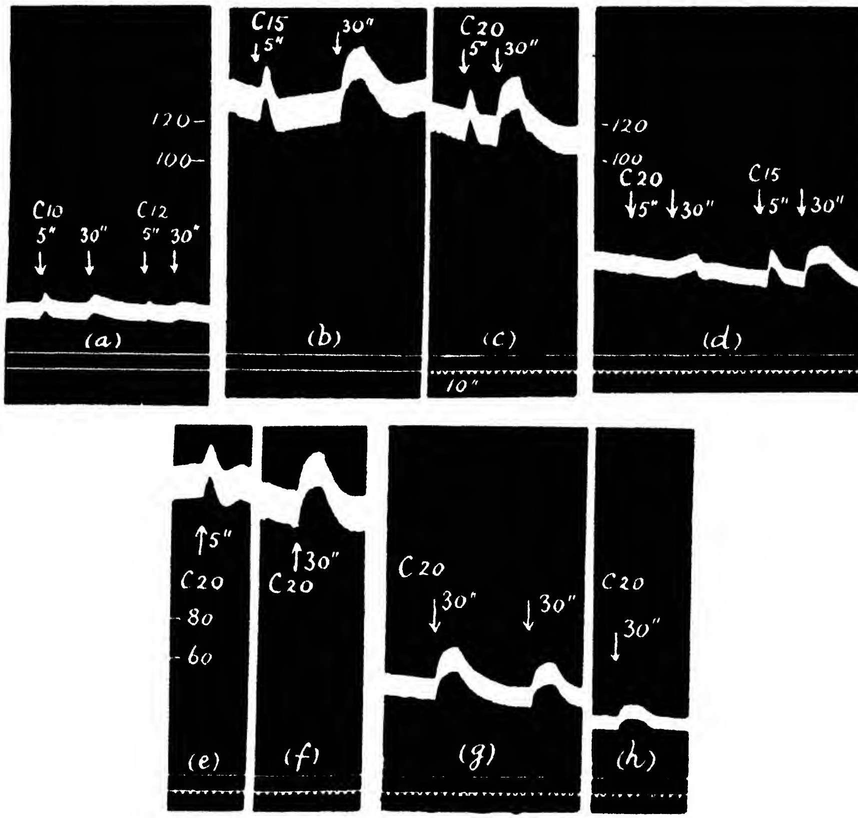

Fig. 5. The record shows in *a* that, in the absence of adrenaline, the weakest effective stimulus was obtained with coil at 10 to 12 cm. After the addition of adrenaline, stimulation at 15 or 20 cm. produced constriction although the tone was much higher (*b* and *c*). In *d* the tone was lower; stimulation at 20 cm. was almost ineffective, but at 15 cm. effective. Further addition of adrenaline now made stimulation at 20 cm. effective (*e* and *f*), and this stimulation remained effective after the adrenaline addition was stopped so that the tone fell in *h* to what it was in *a*.

The part which adrenaline plays in producing these changes is better understood from consideration of such an experiment as that illustrated in Fig. 6, in which both dilator and constrictor effects followed sympathetic stimulation. In Fig. 6*a* and *b*, as already described, the same feeble dilator response to a powerful stimulus was observed at different

times when pituitary extract had been added to the circulating blood. This response was the same as that observed before the addition of pituitary extract when the pressure in the aortic cannula was as low as 30 mm. After the observations in the presence of pituitary extract had

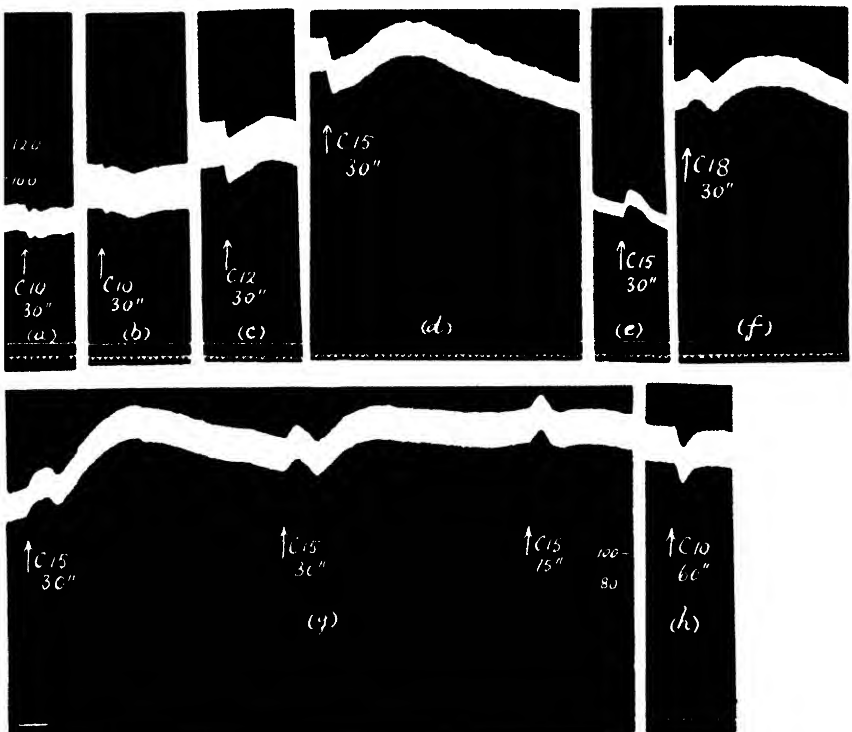

Fig. 6. The record shows at *a* and *b* the feeble dilator responses evoked by sympathetic stimulation with coil at 10 cm. when pituitary (posterior lobe) extract is present in the circulation; the record at *b* was 1 hour later than at *a*. Adrenaline was then added to the circulating blood and the records show the gradual change from a dilator to a constrictor response to sympathetic stimulation. In *e* a pure constrictor response is shown at the same level of tone as existed at *a*. In *g* note the decreasing effect of successive stimulations.

been made, some of the blood was removed and replaced by other blood of the same original bulk, and adrenaline was slowly added to the venous reservoir. Fig. 6*c* shows that with coil at 12, a greater dilator response was then obtained. In Fig. 6*d* the addition of adrenaline was stopped, and stimulation (coil at 15 cm.) produced dilatation followed by slight

constriction. When the tone had fallen low, the same stimulation as the last produced constriction (Fig. 6*e*); this effect, it may be noted, was obtained at the same height of pressure as the dilator response seen in *a*, though stimulation for 30 sec. at coil 15 was at that point without effect. The addition of adrenaline was then begun again, and although the tone was higher, the main effect of stimulation was constriction (Fig. 6*f*). Between Fig. 6*f* and *g* the adrenaline addition was once more stopped and restarted, the effect of the first three stimulations after the restart being shown in *g*. The degree of constriction was progressively less with each stimulation, and the results indicated that the constrictor mechanism was fatigued by successive stimulations.

Discussion.

Evidence has been presented (1) that, in the perfused preparation, stimulation of the lumbar sympathetic chain in the dog leads to vaso-dilatation, (2) that the magnitude of the vascular response to sympathetic stimulation, whether constrictor or dilator, is increased by the presence of circulating adrenaline. The preparation which was used enabled the changes produced by sympathetic stimulation to be observed in the main vessels from the abdominal aorta downwards; hitherto the changes have usually been observed by means of a plethysmograph which, when applied to the leg, records little of the volume above the knee and indicates the response of the smaller vessels only. I have made one plethysmograph experiment, using a dog anæsthetized with chloralose; the viscera were removed and the lumbar chain was stimulated; when adrenaline (1 in 100,000) was infused at a steady rate into the external jugular vein so as to maintain a high arterial pressure, the stimulation was observed to produce vaso-dilatation in the limb.

The success of the perfusion experiments in demonstrating sympathetic vaso-dilator fibres appeared to depend mainly on the observation that the presence of adrenaline in circulating blood augments the response to sympathetic stimulation. In the course of preparing the hind part of a dog for perfusion, the circulation is completely absent for a period varying from 30–50 min., and during this period of anoxæmia all adrenaline present in the tissues (whether at the terminations of the sympathetic nerves or elsewhere) is probably destroyed. Whether this be true or not, the fact remains that when the perfusion is begun and continued for an hour or more with defibrinated blood alone, stimulation of the lumbar sympathetic is almost without effect, whatever the strength

of stimulus applied. This observation is in complete conformity with the previous observations [Burn, 1930 and 1932] that the constrictor action of tyramine and ephedrine, substances which act on that part of the sympathetic nerve ending which does not survive degeneration, is in the same circumstances insignificant. The addition to the circulation of pituitary (posterior lobe) extract does not modify these responses, but when adrenaline is added, the responses become much greater. Tyramine and ephedrine exert a much greater constrictor effect, and in those dogs in which the response to sympathetic stimulation is constrictor, the constrictor response becomes greater as well.

This dependence of the magnitude of the response to sympathetic stimulation on the circulating adrenaline has made easy the demonstration of vaso-dilator fibres. For when adrenaline was added to the circulating blood, the conditions produced were such as to favour the demonstration of vaso-dilator effects, if any were to be obtained. The addition of adrenaline may be supposed to have increased the efficiency of the constrictor fibres and of the dilator fibres to the same extent, but, since it raised the vascular tone, it facilitated the vaso-dilator responses and made more difficult the appearance of the vaso-constrictor responses. It is this circumstance, favouring vaso-dilatation and opposing vaso-constriction, which enabled vaso-dilator fibres to be demonstrated, for under most other conditions the preponderating effect of vaso-constrictor fibres makes the demonstration impossible.

Perhaps the most distinguished school which has till now rejected the evidence for the existence of sympathetic vaso-dilator fibres is that of Cannon. Yet the direct demonstration of the existence of these fibres, or rather of the fact that sympathetic stimulation produces vaso-dilatation, at once affords an explanation of the puzzling results which Bernard Cannon [1931] found to follow the piecemeal removal of the entire sympathetic system. He observed that on each occasion when large portions of the sympathetic in the dog were extirpated, there was a fall of general blood-pressure on the following day, followed by recovery and a supernormal rise to a maximum on the sixth to tenth day. Apart from the immediate effect, the main effect of removal of portions of the sympathetic was not a fall, but a striking rise of pressure, which Bernard Cannon suggested was due "to an over-compensation by the remnant of the sympathetic system at each step, except the last, for the part that has gone." The demonstration of vaso-dilator fibres affords an alternative explanation. The immediate effect of operative removal of portions of the sympathetic system must be stimulation of the peripheral

neurones severed from their central connection; the fall of blood-pressure on the day after the operation may then have been due to stimulation of vaso-dilator fibres by the injury. The supernormal rise on the following days may have been due to the removal of the normal vaso-dilator control exerted by the portion of the sympathetic removed. To those accustomed to conceive of the sympathetic control of the vessels as a mechanism for keeping the blood-pressure high, such an explanation may not readily appeal, yet once the demonstration of the existence of vaso-dilator sympathetic fibres is accepted, it follows that the sympathetic system must act as a mechanism not only for raising, but also for lowering the blood-pressure, and that preconceived ideas of what will happen when the sympathetic is removed must be modified. The experiments of Bernard Cannon may, in fact, be a demonstration that in the dog the sympathetic vaso-dilator fibres are of more daily importance than the vaso-constrictor fibres; it may be during moments of stress only that the constrictor fibres are called into play.

It has been suggested that if the view is correct for which Loewi [1921] first adduced important experimental evidence, though it was originally propounded by Elliott [1904], namely that the sympathetic fibres exert their effect by the liberation of small amounts of adrenaline, there may be no essential difference between vaso-constrictor and vaso-dilator fibres. Since small amounts of adrenaline cause vaso-dilatation, and larger amounts vaso-constriction, it is possible to suppose that the effect of stimulating the same nerve fibre may be to produce either vaso-dilatation or vaso-constriction according to the conditions of stimulation. A weak stimulus might liberate a small amount of adrenaline and lead to vaso-dilatation; a stronger one might liberate a larger amount and cause vaso-constriction. The results described in this paper do not conform with this suggestion. If the results illustrated in Fig. 3 be examined, the sequence of dilatation, constriction and dilatation follows the stimulation with coil at 10 cm.; this sequence can only be understood if it is assumed that there are two sets of fibres which are stimulated simultaneously, the one dilator and the other constrictor, so that the result of stimulation is the algebraic sum of the two effects; if there were not, the dilatation following the constriction would not be seen. Furthermore, as illustrated also in Fig. 3 (and other figures), the effect of employing a stronger stimulus was regularly observed to favour the appearance of vaso-dilator effects; to obtain vaso-constriction, the weaker stimuli applied for a longer time were most suitable.

The effect of adrenaline in augmenting the response to sympathetic

stimulation is easy to understand if the truth of the view is granted that sympathetic stimulation results in the release of adrenaline. This view has now received the support of the experiments of Cannon and Bacq [1931], in which stimulation of the pilomotor fibres in the cat was shown to cause a rise in the rate of the denervated heart. The authors postulate the existence of a substance "Sympathin" which is released in the blood after sympathetic stimulation.

If it is true that sympathetic stimulation releases adrenaline, then it is evident that a store of adrenaline must exist at the sympathetic nerve ending ready for release; the feeble response to stimulation of the lumbar chain in the perfused preparation may then be attributed to the destruction of this store during the period of anoxæmia between the death of the animal and the beginning of the perfusion. With the addition of adrenaline to the circulating blood, the store is gradually replenished, so that after a time the stimulation of the lumbar chain produces a much greater effect.

This, however, is merely one possible explanation of the part played by adrenaline in increasing the efficiency of sympathetic stimulation.

The question remains whether the effect exerted by the adrenaline in the perfusion experiments is peculiar to such experiments, or whether it can be demonstrated in the animal. I have already made some experiments on cats in which both suprarenals were removed on one day, and the effect of sympathetic stimulation on the vascular response was observed on the following day. Evidence was obtained that the addition of adrenaline to the circulating blood again augmented the response to sympathetic stimulation. Before publishing these results, it seems desirable to make a longer investigation than has yet been possible.

Summary.

1. The existence of vaso-dilator fibres in the lumbar sympathetic chain of the dog has been demonstrated.

2. It has been shown that both the magnitude of the vaso-dilator response and the magnitude of the vaso-constrictor response to sympathetic stimulation are dependent upon the amount of adrenaline in the circulating blood.

I wish to thank Mr H. W. Ling for his help in the experiments.

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IDENTIFICATION OF THE GAMMA EXCITABILITY IN MUSCLE.

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WHEN a muscle is excited through large fluid electrodes it may exhibit at least two excitabilities characterized by very different time relations (excitation times)[1]. Lucas [1907–8], who was the first to demonstrate this, called γ the excitability resembling nerve in its excitation time, and α the excitability which was much slower. He believed γ to be due to the intramuscular nerve twigs, since in addition to its resemblance in excitation time it was absent from the nerve-free part of the sartorius and was abolished by curare: α he supposed to be the excitability of the muscle fibres themselves. Until the last year or so this view was strongly opposed by Lapicque, who used a different electrode system, never obtained α, and concluded that nerve and muscle have the same short excitation time, and that Lucas's results were due to experimental errors.

The work of Jinnaka and Azuma, Davis, and Watts shows clearly that the excitation time of muscle diminishes very greatly with diminution in size of the electrodes employed, though with nerve this effect is small; hence by the use of smaller and smaller electrodes the muscle excitation time will diminish much faster than will that of the nerve, and consequently the two will approach.

Now if the detection of two excitabilities rests upon the observation of a kink in the strength duration curve, it is necessary that the two curves in question should in the first place meet and in the second place meet at a significant angle. But if the two excitation times are nearly the same,

[1] Since Lapicque has insisted that the name "chronaxie" is not to be applied to the constant derived from strength duration curves unless they fit an empirical formula which he has introduced, I have recently proposed [1932] that Lucas's term "excitation time" be used to designate the constant in all cases whatever, and thus the chronaxie is a particular excitation time which satisfies Lapicque's requirements.

there is very little chance of the curves meeting, since one is apt to lie completely above or below the other, and if intersection does occur it is little likely to be noticed on account of the insensitiveness of the usual method of representation.

From these considerations it is clear that, if we wish to be sure of the presence of both α and γ excitabilities, it is important to use sufficiently large electrodes for the α excitation time to be long, for γ resembles nerve in that its excitation time is relatively independent of electrode size.

With this in mind I have recently reinvestigated the question of whether the α phenomenon represents a separate excitability, or whether it is due to some error or abnormality [1930]. Using fluid electrodes with interpolar length of 1 cm. or more, the α curve can be obtained from any voluntary muscle, and all the evidence points to its being a normal constituent of the tissue. The question then arises, if the α excitability is a normal muscle element, what is the γ? Is it a different normal muscle element, is it the same element but excited differently (*e.g.* when excitation is elicited from the middle, not the end of a muscle fibre as suggested by Moore and Brücke [1931]), is it nerve, or is it junctional tissue? It is this question which the present investigation sets out to answer, but at the outset one point must be made clear. The γ excitability to be identified is that element which gives a γ curve in experiments with uniform electric fields when the irregularities at the terminations of muscles are screened [cf. Rushton, 1930, p. 331]. The fact that a curve with γ time relations may be obtained with the use of a pore electrode does not necessarily mean that the substance there excited was the same as that to be investigated in the present paper. It is hoped that this study of comparatively simple and regular cases may throw some light on the difficult cases of non-uniform fields.

Localization of the γ excitability.

(*a*) *The size of the γ substance.*

By γ "substance" is meant the tissue which is directly excited by a γ stimulus and whose time relations characterize the γ curve. As a first step towards identifying this substance with some definite histological entity, we proceed to enquire as to the size of the substance, whether for instance it be small or large compared with 1 mm. Light upon this matter is thrown by the relation between threshold and position of the electrodes. This relation was determined upon two species of frog, *R. pipiens* (U.S.A.) and *R. temporaria* (England). The results in each case lead to the same

conclusions, but those with *R. temporaria* are more straightforward and will be considered first.

In order to expose any given region of the muscle to a uniform electric field whilst keeping each end of the muscle equipotential, the excised sartorius was held by its ends on the floor of a rectangular trough filled with Ringer's fluid [Rushton, 1927, Fig. 1]. The pelvis was fixed and the tibial tendon attached to a light tension lever. Silver plates which fitted the cross-section of the trough (except for a gap below for the muscle) served as electrodes, and could be moved at will over any part of the muscle. Since there was a uniform field between the plates and an equipotential region beyond, they fulfilled the conditions of the present investigation so long as they did not move to the extremities of the muscle and extend the field to the irregularities there. In series with the electrodes was a non-inductive resistance of 10,000 ohms. Current strengths were controlled by a high resistance potentiometer used in conjunction with a voltmeter; durations of current by a Lucas pendulum. In order that the silver electrodes should be non-polarizable they were coated with chloride in the usual manner, but in this condition they appeared to have some toxic properties.

When freshly chlorinated electrodes were placed in Ringer's fluid just above the muscle, it was found that spontaneous rhythmic contractions resulted. Sometimes these started within a few seconds of applying the electrodes, sometimes not until current had been passed between them. The spontaneous contractions when once started continued a long time in spite of removal of electrodes and changes of fluid. This difficulty in former experiments led me to abandon the Ag-AgCl type of electrode and use Zn-$ZnSO_4$ Agar-Ringer. For the present experiment it was necessary to use plate electrodes, and so I returned to the question of these spontaneous contractions. The electrodes were made of pure electrolytic silver, the soldered connections were coated with paraffin wax, and after chlorination the electrodes were washed for an hour in running water. The contractions still appeared, and it seems probable that they are due either to the AgCl, or to the Ag^+ ion which, according to the theory of the non-polarizable electrode, must be present in the solution round the electrode in appreciable concentration. Seeing how commonly this type of electrode is used it might seem remarkable that the present observation is not of general occurrence, but in the first place there is often a wad of cotton-wool or clay between electrodes and tissue, and in the second, nerve is the tissue more often investigated, and this tissue is not much affected. I have never noticed any effect upon the nerve in these circumstances which may perhaps be attributed to the thick connective tissue sheath. Feng and Gerard [1930] have shown what part this sheath may play in withstanding the diffusion of substances, and if a nerve trunk is left overnight in 2 p.c. $AgNO_3$, and the sheath removed next day, after washing, the fibres within are not stained.

In the present experiment, therefore, the electrodes after chlorination were coated with Agar-Ringer, in which condition they were found never to give rise to spontaneous twitchings.

In the apparatus described above, it is the E.M.F. of the exciting circuit which is read, but what we require to know is the current or potential gradient in the solution. In order to obtain the relation between these two it is necessary to calibrate the apparatus for various positions of the electrodes. Both physical and physiological calibrations agreed in showing that the variable fluid resistance was so small a fraction of the fixed resistance of the circuit that the current was always proportional to the E.M.F. correct to 1 p.c.

The physical calibration was made either directly by placing a milliammeter in series and reading the current flowing in the various cases, or by finding the resistance of the electrode system in an A.C. bridge (1000 cycles) and calculating the variation in the final circuit. In the physiological method a sciatic nerve bent double on itself was substituted for the muscle in the trough. The anode was placed over the nerve a few millimetres from the bend and the cathode placed at various distances away so that the bend always lay in the region between the two electrodes. In this arrangement the physiological cathode always lies at the bend, the interpolar length remains constant, and hence the threshold always corresponds to some definite value of the current in the trough. It was found that the threshold E.M.F. was independent of the distance apart of the electrodes.

We may therefore take the voltmeter readings as proportional to the potential gradient in the trough.

The experiment was performed as follows. The sartorius was set up as described with the deep surface uppermost, and the cathode was placed over the muscle near the pelvic end. The anode was placed near the other extremity of the muscle, and a strength duration curve taken. The γ curve was always prominent, and a stimulus duration of 10σ usually was sufficiently short to avoid confusion with the α threshold. Some shorter duration, usually 1 or 2σ was taken and used throughout the experiment, and the threshold was found for different positions of the electrodes. Fig. 1 shows the results of one experiment, where ordinates give the voltmeter readings of the threshold (size of "point" gives limit of error) and abscissæ show the position of the variable electrode.

Consider first curve A, which corresponds to a cathode fixed at O at the pelvic end of the muscle and movable anode. At first, diminution in interpolar distance is unaccompanied by any threshold change, but soon the curve rises smoothly to become vertical at an electrode separation of 17 mm. This result is very regular and can be repeated accurately. In many experiments (cf. Figs. 3 and 9) the vertical part of the curve is met by a new horizontal line corresponding to an excitability unaffected by further movement of the anode until the electrodes lie only 2 or 3 mm. apart. With regard to this horizontal part of the curve it may at once be stated that it is the α excitability, and we find again the well-known

observation that (with large electrodes) α alone is present at the pelvic extremity. In order to learn whether it be possible that the γ substance be small compared with 1 mm., let us assume this to be the case and interpret the results of Fig. 1 in terms of this hypothesis. It is clear that, if the most excitable γ element lies somewhere between the electrodes,

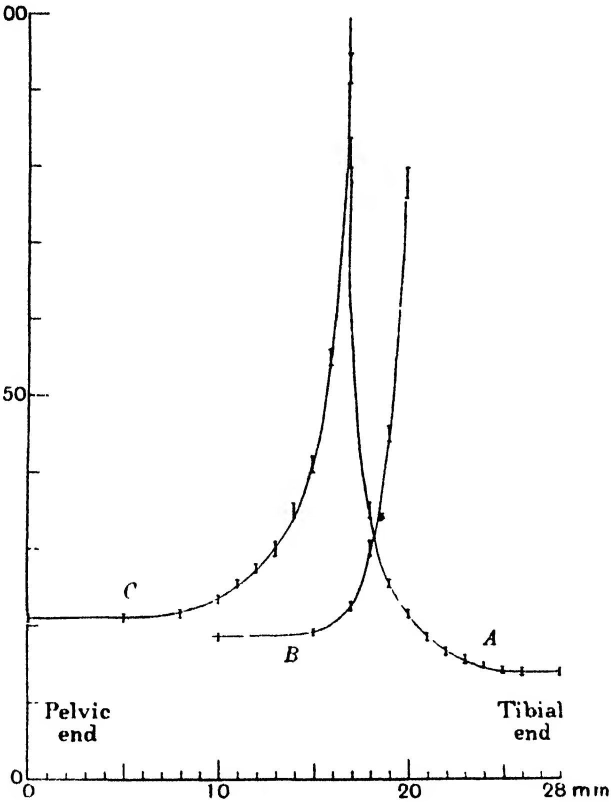

Fig. 1. Strength-length curve (*R. temporaria*). Abscissæ length in millimetres, ordinates current strength in arbitrary units. *A*, *B*, *C*, see text.

the observed threshold will depend simply upon the current in the neighbourhood of this γ element, and hence will be unchanged by movement of the electrodes until one of them passes over this element and excludes it from the field, when the threshold will promptly rise to that of the most excitable element now remaining in the field. Interpreting Fig. 1, curve *A*, in this manner, therefore, we must conclude that the most excitable γ elements are situated near the extreme tibial end of the muscle, and that

those with increasing threshold are distributed with surprising regularity as we approach the centre of the muscle; the pelvic half is very inexcitable, since in general no γ excitability is revealed when the anode lies on the pelvic side at the place where curve A becomes vertical. This may not have an air of great probability, but the evidence so far is not definitely incompatible with the hypothesis of small γ elements; by a further experiment, however, we may completely exclude this hypothesis.

Fig. 1, curve B, is the result of repeating the observations on the same preparation, but with the anode now fixed at the point marked 22 mm. and with the cathode at the various positions shown by the abscissæ. On the hypothesis of small γ elements we must again suppose that the variation of threshold is due to the exclusion, one by one, of the most excitable elements from the field, and since curve B rises as we move towards the tibia, it follows that in this case the most excitable fibres lie on the pelvic side. But this introduces the contradiction that, in that region of abscissæ where the two curves are both sloping markedly and in opposite directions, the γ elements must be assumed for A to be more excitable towards the tibia, for B, more excitable towards the pelvis. Specifically, in the region between 17 mm. and 20 mm. a movement of the anode (curve A) causes a sharp change of threshold, showing that the γ elements in the neighbourhood of this electrode are more excitable than any other elements in the field. But if instead of moving the anode the cathode is moved (curve B), there is also a sharp threshold change, indicating that the elements in the cathode neighbourhood are the most excitable. Since the two conclusions are necessarily incompatible we are left no option but to reject the assumption that the γ elements are small, and to conclude first that the observed thresholds are affected by the length of the γ substance exposed to the field, and second that the lengths in question must be at least of several millimetres.

(b) *The position of the γ substance.*

Granted that the γ substance has extension of several millimetres it is not surprising to find that the threshold depends upon how much of this length is exposed to the stimulating current. Experiments upon the relation between interpolar length and threshold have been made by a large number of workers, who have agreed that the curve obtained resembles the strength duration curve (thresholds plotted vertically in each case) in that it becomes asymptotic to the vertical axis and to a line parallel to the horizontal axis but above it. An example of a strength-length curve obtained upon nerve by the method of the present paper is shown in Fig. 2

of a former paper [Rushton, 1927], and some results of earlier workers are shown there in Fig. 3.

Now, in the present case, we have seen that the γ substance must have several millimetres extension, and thus it must have a cylindrical structure, for no other kinds of excitable structures of that size are present in the sartorius. But, as I have attempted to show [1927], the very cylindrical structure will account for the exact form of the strength-length curve on the simplest physical assumptions. Hence it is natural to suppose that the γ fibres will exhibit a strength-length curve of the usual shape. This is seen in Fig. 1 to be the case. As the electrodes approximate, no matter which is moved, the threshold rises and curve A, where the cathode is fixed, is seen to resemble closely the corresponding curve for nerve shown in the former paper. It is not to be supposed that the γ fibres necessarily stretch all the way between the electrodes in every case, but fibres must run towards the tibia at least as far as the point where the curve A becomes horizontal, for if they ended at some earlier point we could not account for the continued fall in threshold as the anode moved from this point to the 25 mm. mark. By the same argument the γ fibres must run towards the pelvis at least as far as the point where the curve A becomes vertical. But, whereas it is possible that the fibres extend tibially even further than the point where curve A becomes horizontal, it is not possible that these fibres run further towards the pelvis than the vertical asymptote. Both from theory and experiment with excised nerves it is found that the curve only becomes vertical when the interpolar length becomes infinitesimal, and we may therefore conclude that the γ fibres stimulated in curve A run towards the pelvis only as far as the vertical asymptote; between this point and the pelvis the length of fibre is infinitesimal, hence the fibre either here ceases altogether, or else turns and runs no further in the pelvic direction. From this analysis we may, therefore, conclude that the histological elements which give rise to the γ curve A, are fibres which start at a fixed point 17 mm. and run in the tibial direction for at least 8 mm.

When the experiment of Fig. 1, curve A, is repeated with the current reversed and the cathode now fixed at the tibia, curve C, Fig. 1, results. In this case the cathode was kept fixed at 28 mm. and the anode moved to points as indicated by the abscissæ. The curve obtained is in all respects similar to A and may be shown like A to be due to the change in interpolar length and not to the exclusion from the field of small excitable structures. All the former analysis of A applies equally to C, and we must conclude that a second series of γ fibres exist starting at a definite point and running

in the opposite direction. We note, moreover, that the vertical asymptote for curve A is the same as for that of curve C, whence we conclude that at this point, which we may call the "origin" of the γ fibres, they start and run, some towards the pelvis and some towards the tibia for distances of at least 8 mm. We must also accept the remarkable fact that when the whole muscle is placed in a uniform field (parallel to the axis of the muscle) only those γ fibres which lie on the anodic side of their "origin" respond, whichever the direction of the current.

(c) *Direction of γ fibres.*

One deduction from the cylindrical structure of the γ fibre is the form of the strength-length curve; this as we have seen is experimentally verified. Another deduction [Rushton, 1927] is the relation between threshold and the angle between fibre and direction of current. It appears that, if a straight fibre is immersed in a uniform electric field at any angle, it will be excited proportionally to the component of the field resolved in the direction of the fibre. Experimentally this has been found by many workers both in muscle and nerve, and results obtained with the apparatus of the present investigation are given for nerve [Rushton, 1927, p. 371], for muscle [1930, p. 332], where former work is discussed. In particular the method of plotting in polar coordinates has considerable advantages, as the relation between angle and threshold appears as a straight line perpendicular to the direction of the fibres. It is, therefore, clear that we may find the direction of unknown straight fibres by obtaining this line from the threshold-angle results in polar coordinates, and drawing a perpendicular to it.

Experiments exactly upon these lines have already been described [Rushton, 1930], and Fig. 14 of that paper shows a set of results with the sartorius, where it is seen that the γ fibres (in contradistinction to the α fibres whose thresholds lie within the heavy curve) run in a great many directions. This result is always obtained, and similar threshold-angle curves are shown here in Fig. 5 (upper half) and in Fig. 9 (bottom). The actual curves vary greatly from one preparation to another, but always agree in that the γ fibres run in a great many directions. The complexity of the results with the sartorius makes a detailed analysis unprofitable, but further light on this question will be thrown by the comparatively simple case of the sterno-cutaneous strip, considered in a later section of this paper. For the present suffice it to remark that the γ fibres are by no means restricted to the direction of the muscle fibres, but run in many different directions and vary greatly from one preparation to another.

COMPARISON WITH HISTOLOGY.

There are several other lines of investigation which may be employed to identify further the γ fibres, but since the foregoing analysis would suggest very strongly that these fibres are not muscle but nerve fibres (even if this conception had not already been supported by the evidence of Lucas), it will be convenient to consider this hypothesis at once. In this way it will first be shown that the results so far considered fit in detail, and then the further analysis may be presented in connection with the histology of the nerve fibres, so that the correspondence may be more directly appreciated.

We have seen from the evidence of Fig. 1 that the γ substance is in the form of fibres which start from a point, their "origin," and run some towards the pelvis, some towards the tibia for a distance of about 8 mm. or more. If all the muscle fibres ran from one end of the sartorius to the other, this evidence would exclude the muscle fibres from being identified with the γ substance, but since some fibres certainly end in the body of the muscle, it might conceivably happen that these were more excitable than the others, and thus account for the observation. However, the evidence concerning the *direction* of the γ fibres excludes this possibility, and microscopic examination does not show any large number of muscle fibres ending at the γ origin, but it does show that the γ origin corresponds exactly with the point of entry of the nerve trunk.

This correspondence cannot be regarded as a coincidence. The localization can easily be made in the fresh preparation during the actual stimulation, correct to $\pm$ 0·5 mm., and in all of a large number of experiments with different kinds of technique the correspondence has been exact within these limits. Fig. 2 shows quite a different form of apparatus similar to Lucas's. It is less accurate and more troublesome than that already described, but it is interesting as affording a control upon the other method, since in this case "deformation of current" is of quite a different kind from that where the muscle is completely immersed, and also because the zinc electrodes are separated from the muscle by side tubes filled with Agar-Ringer, so that poisoning and potential discontinuities are eliminated. The interpolar length of the muscle is the part in air between the two liquids. This was varied by opening the tap to cistern A, and for each position of the liquid the threshold voltage and also the A.C. resistance of the circuit were measured (1000 cycles). Fig. 3 shows the muscle (*R. pipiens*), stained for nerves by May's method, and also

the strength-length curve with cathode fixed at the pelvis. The strengths are proportional to the calculated current intensities. It is seen that, by this method also, the vertical asymptote coincides with the point of nerve entry. The horizontal line to the left of the vertical was found to be due to the α excitability. Fig. 4 shows the curves of Fig. 1 together with the corresponding preparation stained for nerves by May's method, and we see the verification of the earlier deductions that fibres start at the point

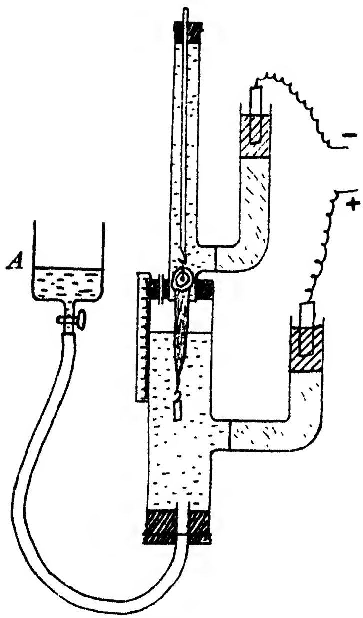

Fig. 2.

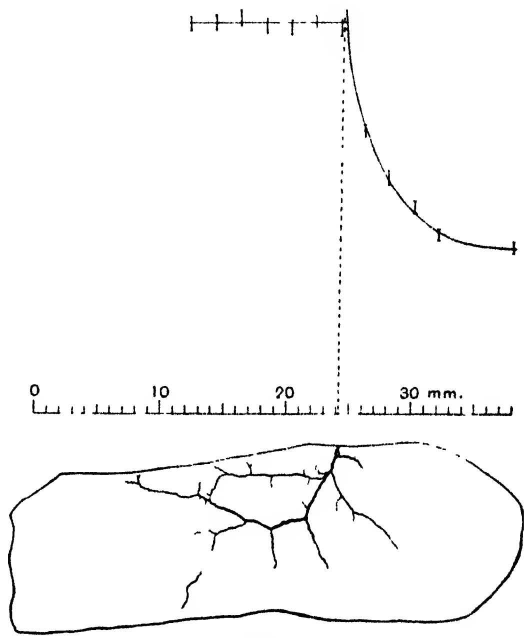

Fig. 3.

Fig. 2. Second form of apparatus.

Fig. 3. Strength-length curve from apparatus of Fig. 2. Sartorius muscle (*R. pipiens*) drawn to the scale of the horizontal axis.

"17 mm." and run for at least 9 mm. to the left. With regard to the fibres running to the right, they should run at least 4 mm. further than appears in the tracing. This discrepancy is probably due to the fact that the peripheral nerve twigs do not always take the stain very well, and it is easily possible that some long fine branches may run for several millimetres further to the right without appearing in the tracing. As a result of the foregoing experiments, therefore, we may conclude that

the nerve fibres fit the requirements of the γ substance to the following extent.

(*a*) They are in the form of fibres.

(*b*) These enter the muscle at the exact point where the γ fibres start.

(*c*) They run in both directions a distance of some 8 mm. measured along the axis of the muscle.

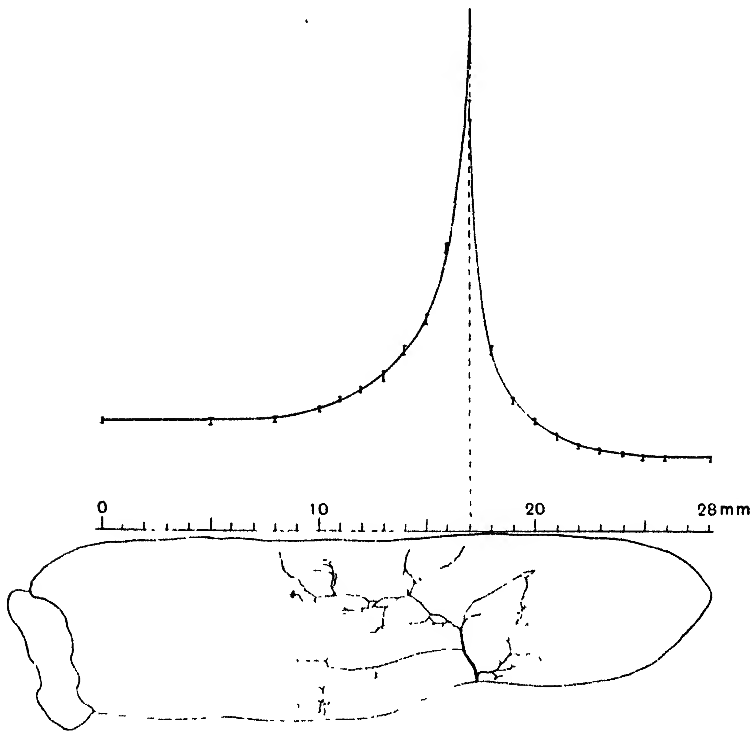

Fig. 4. Strength-length curves from Fig. 1 and sartorius muscle (*R. temporaria*) drawn to scale.

(*d*) They run in many directions.

(*e*) They are absent from the pelvic extremity.

(*f*) They have the same excitation time as nerves.

This identification may be yet considerably strengthened by experiments with the sartorius of the American green frog and the sterno-cutaneous of the American bull-frog, but before describing this work it will be well to consider the critical question: "Are the γ fibres undetectable after curare?"

CURARE.

I do not wish to discuss at any length the action of curare until a later publication, but if γ fibres are nerves the abolition of their action by the drug is so obvious a consideration that it clearly deserves investigation in this place. Without the necessity of entering into details, however, it may

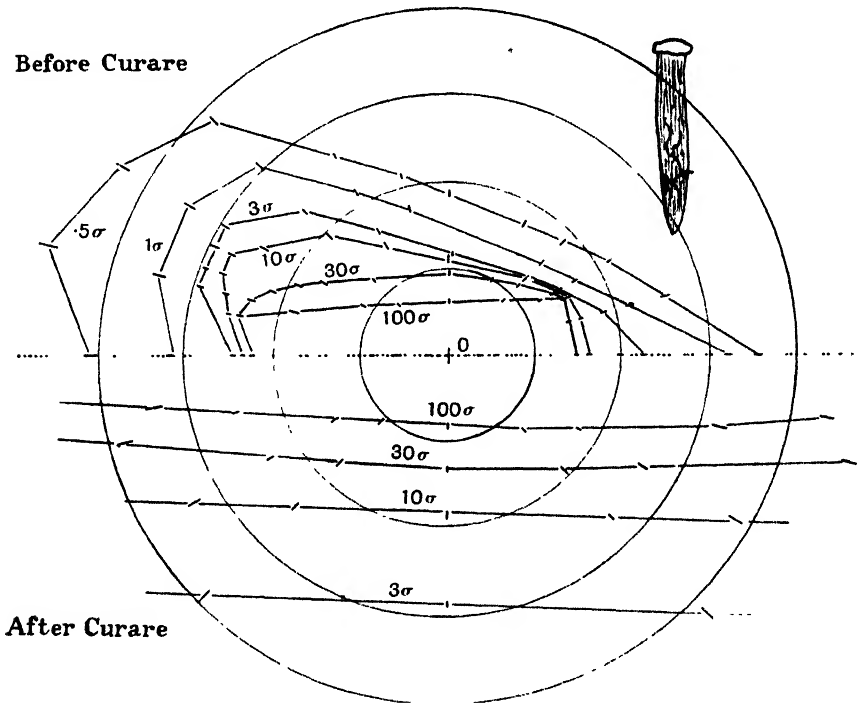

Fig. 5. Threshold-angle results for various durations of stimulus. Results before curare above horizontal, results after curare reflected below. Distance of "point" from O is proportional to threshold, direction of line from "point" to O is the direction of the current with respect to the muscle shown diagrammatically (*R. pipiens*).

at once be stated that no case was ever encountered where the γ excitability remained after curarization. These observations upon the action of curare are in complete agreement with the present hypothesis, but they fit equally the theory of Lapicque that the γ substance may be muscle, and that the γ excitability vanishes after curarization, not because the excitable fibres are no longer operative, but because their excitation time has lengthened and no longer has the γ time relations. The following experiment decides against Lapicque's explanation.

The threshold-angle curve of a sartorius was determined at various durations of current flow, and the results are shown in Fig. 5 plotted as usual in polar coordinates. The upper half of the circle shows the results before curare (cathode towards pelvis), the repetition of the same after curarization is shown for clearness reflected about the horizontal diameter, and they thus appear below.

These lower results are, therefore, uncomplicated by the irregularity of nerve structure, and appear exactly as would be expected—a set of parallel straight lines, indicating fibres running in the direction of the muscle fibres.

Now since curare cannot alter the direction of a fibre, the upper results of Fig. 5 should also be a set of horizontal parallel lines if Lapicque were right, and the fibres excited initially were the same as those after curarization. But these upper results indicate quite a different set of fibres running in various directions in the way that has already been shown to be normally the case with γ fibres. It is, therefore, clear that the action of curare upon the γ fibres is not to alter their time relations as Lapicque suggests, but to remove their activity altogether and to leave a new set of fibres (α) with different time relations, and a different direction, namely the direction of the muscle fibres. The identification of γ with nerves, therefore, is strongly supported by the effect of curare, which was always found to have abolished the action of the γ excitability at the moment when excitation through the nerve failed.

Sterno-cutaneous muscle.

It was remarked in a former paper [Rushton, 1930] that the irregularities which obscure results with the sartorius can be largely overcome by using a strip of sterno-cutaneous muscle, and Fig. 12 of that paper shows some threshold-angle results. Fig. 6 of the present work shows the results of a similar experiment with the tracing of the corresponding muscle strip (on the right) stained to show its nerve. The experimental details are given in the former publication, and it will here suffice to say that the strip of muscle was cut from the medial border of the sterno-cutaneous of a bull-frog, by a clean snip parallel to the muscle fibres. The threshold-angle results were obtained in the usual way, and then the preparation was stained by May's method, mounted in gelatine and a tracing made from an optical projection.

With regard to Fig. 6 we notice that the excitability represented by the horizontal lines changes threshold greatly between 100σ and 30σ

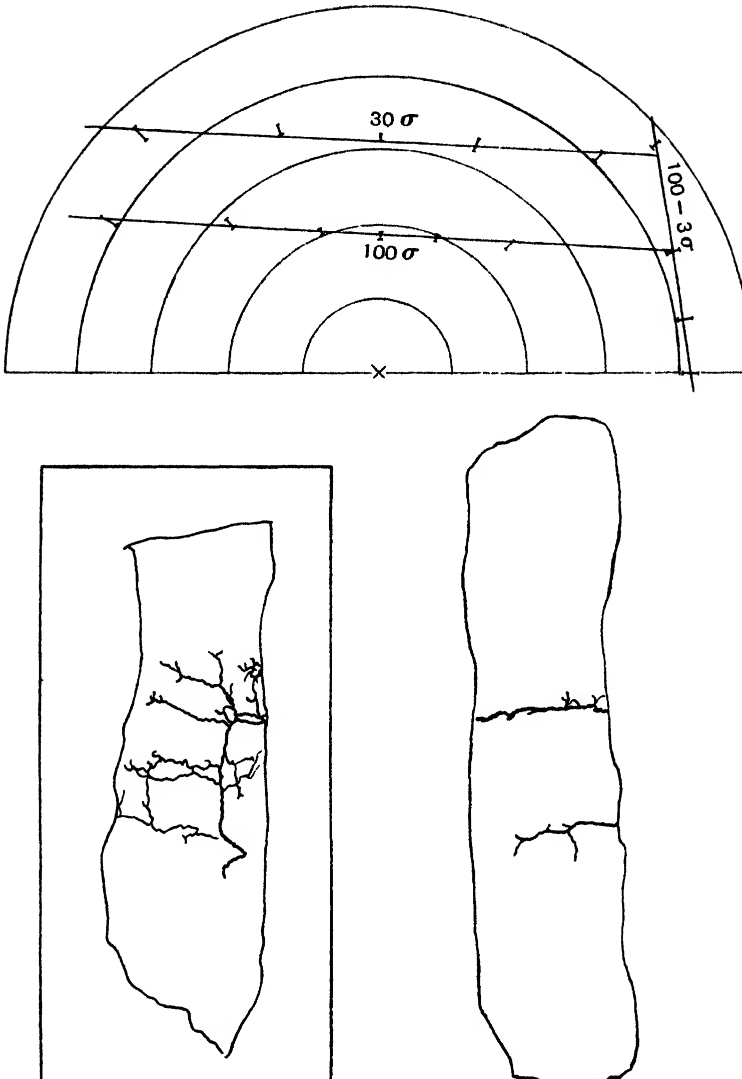

Fig. 6. Threshold-angle results for the sterno-cutaneous strip, shown below (right). Sterno-cutaneous strip from another frog (left). Plotting as in Fig. 5 (bull-frog).

duration, and hence must be α which again corresponds to fibres in the direction of the muscle, as in the case of the sartorius. The other excitability, on the contrary, has a utilization period of less than 3σ and is therefore γ which is seen to make an angle of 75° with this direction. It is clear from the tracing (right) that the directions of the muscle and the nerve fibres are very nearly perpendicular to those of the α and γ lines respectively. Other experiments confirm this; the angle between the γ and α lines varies from preparation to preparation, but there is always a corresponding variation in the angle between nerve and muscle.

On one occasion the γ line instead of being straight was quite curved, indicating fibres in many directions. When this preparation was stained and examined it presented the appearance shown traced in Fig. 6 (left).

The action of curare is also clearer here than in the case of the sartorius, since the pronounced difference in the directions of the γ and α fibres makes it easy to see that the γ are removed and α left [cf. Rushton, 1930, Figs. 11 and 12]. The results of the experiments with the sternocutaneous, therefore, bear out in all particulars the less satisfactory results with the sartorius, and confirm and extend the correlation of the γ excitability and the nerve twigs.

Excision of nerve twigs.

An obvious if crude test for the present hypothesis lies in the effect of removing by dissection some of the principal intramuscular nerve branches in the sartorius. This is much easier with *R. pipiens* than with *R. temporaria*, since, with the former, the nerves after they enter the muscle lie very superficially and but slightly bound down by connective tissue. This was easily slit with a sharp cystotome and the nerve, lifted by the end with forceps, could then be freed from the subjacent tissue, for several millimetres along each branch, and then cut. This operation removes the nerve with very little damage to the muscle, and when the final stained preparation was examined, the muscle fibres could be seen intact, the nerve fibres cleared from the region of the nerve entry, and the place where they had been, approximately marked by the rows of the pigment cells which follow the nerve branches here very closely.

When this operation is performed and the strength-length curves determined before and after, according to the method of Fig. 1, it is found that the γ excitability initially present is completely removed and that α alone remains. No figure is here given in illustration of this state-

ment, since the initial γ curves are of course normal and the subsequent γ curves being absent cannot be represented.

The experiment, however, gives a very different proof of the identity of γ and nerves.

THE ANALYSIS OF COMPLEX CASES.

If a sartorius is stimulated as in the experiment of Fig. 1 with nearly the whole length of the muscle exposed to the current, we might expect that a γ excitation could be elicited from a great many points among the ramifications of the γ fibres, and that the actual point giving the threshold would be merely that particular place where the excitability was greatest to the stimulus in question. The simplicity of the results so far considered depends upon the fact that the "γ origin," which appears always to be a highly excitable point, in these cases is so much more excitable than any other place, that the only γ excitation which appears at all arises from here. By this I do not mean that it is impossible by any placing of the electrodes to elicit a γ response from elsewhere, since curve *B*, Fig. 1, is a ready instance to the contrary, but when the cathode lies at either extremity of the muscle, then this impossibility arises in all those muscles so far considered. Convenient as this is in simplifying the analysis, it is so singular that we should expect some preparations to exhibit other excitable points. *R. pipiens* nearly always shows two or more such points, and with *R. temporaria*, though this is not so common, the same may often be found.

It is natural to enquire what places are the most excitable in the conditions of these experiments, and the answer must involve two factors:

(*a*) The local excitability of the point in question, *i.e.* the amount of polarizing current required to excite this point.

(*b*) The current distribution in the fibre at the point in question, *i.e.* the proportion of the field current which is flowing in a direction to polarize the fibre at this point.

With regard to (*a*) little can be said. I see no reason why the local excitability could not vary at random from point to point, though actually the variations do not seem very great. Superimposed upon these there is the well-known hyperexcitability of a fibre in the neighbourhood of the cut end after recent section, probably correlated with the catelectrotonic effect of the injury current [Gotch, 1900]. This may in part explain the hyperexcitability of the "γ origin," if we identify γ with nerves.

With regard to (*b*) more can be said than is suitable in this place. The distribution of current in cylindrical fibres is a physical problem which

admits of an accurate solution. I have already published [1928] a general formula which covers all cases with which we may here be concerned (except for three or more fibres branching from a point, which requires a slight extension of the method), but it suffers from two objections. In the first place solutions involving definite integrals do not recommend themselves to some physiologists; in the second, the data in practice are presented in the form of a traced curve (*e.g.*, the course of the intramuscular twigs) while the formula requires it in the form of an analytical expression. I have recently been able to overcome both these objections by a method of solution which gives the results graphically from a simple construction with pencil and scale, and which involves no mathematical technique. This method I hope to publish in the near future and to apply it to the more detailed analysis of γ and other excitability measurements, and therefore certain statements in the present paper will rest merely upon their general probability, waiting for the subsequent work for a more complete justification.

Chief among these are the ideas connected with excitation at the bend of a fibre. If in Fig. 7 the current flows in the direction of the arrow, and the fibre ABC is immersed in this field, the most excitable point will be B, for this is the most cathodic of all the points in the fibre. The sharper the point at B, and the closer the lines AB, CB to the direction of the current, the more excitable will B be; thus in a complex ramification of fibres we should expect sharp bends to be the most excitable points, provided that the current flows into the angle as in Fig. 7. An experimental verification of the idea together with a theoretical treatment has already been published for the case of bent nerve [Rushton, 1928].

Now if the cathode of the trough used for Fig. 1 lies to the left of B, Fig. 7, and the anode approaches B in the direction of the arrow, this anode may be represented as a line which is perpendicular to the current direction and which moves in the direction of the arrow. Thus the interpolar lengths BA_1, BC_1 will be shortened, and hence the usual strength-length curve will be obtained with this difference, that 1 mm. movement of the electrode will cause much more than 1 mm. diminution in BA_1 and BC_1, owing to the foreshortening of the fibre. Thus the strength-length curve obtained will be foreshortened to the same extent, and the threshold plotted against position of the electrode will rise very much more sharply than usual. An example of this will be considered later (Fig. 9).

Before turning to the experimental results there is one more point the consideration of which will elucidate the principal features of these observations. If in Fig. 8 two fibres A, B end at the distance O and both run to

the right for some way, then they will both give similar strength-length curves, A, B, but if B is less excitable than A, curve B will always lie above A and will never be found by ordinary threshold measurements. Suppose now that the whole fibre B is moved to the left (B_1) so that it ends at O_1, then the strength-length curve will similarly be moved an equal distance to the left (B_1), and hence will now cross A and be seen in threshold measurements as a kink in the strength-length curve. We may locate the two points O and O_1 whence the two excitations arose by extrapolating the curves A and B_1, till they become vertical, as is seen at once from Fig. 8.

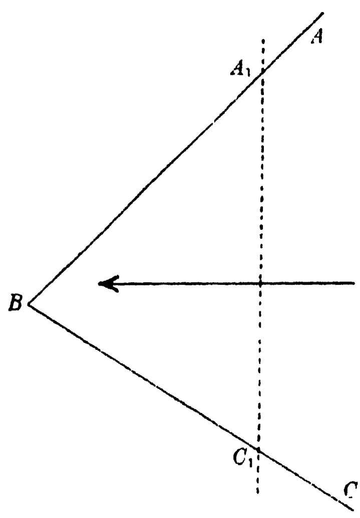

Fig. 7.

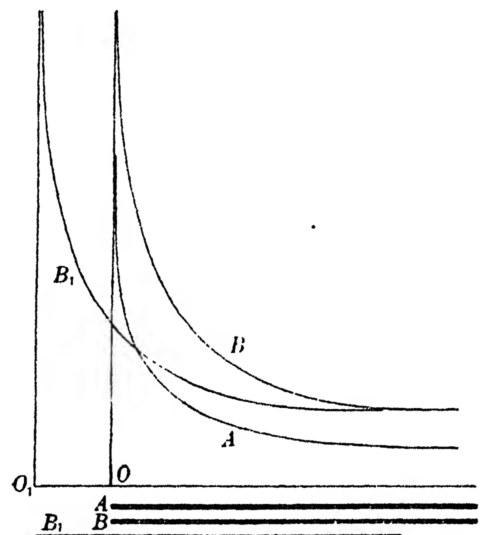

Fig. 8.

To summarize this analysis, therefore:

(a) A kink in strength-length curve signifies two excitable points.

(b) The point whence excitation was elicited for each portion of the curve lies where the extrapolation of this portion becomes vertical.

(c) The foreshortening of each portion of curve as compared with a standard[1] strength-length curve is equal to the foreshortening of the corresponding fibre when projected on to the direction of the current.

[1] The standard curve would be that obtained from a straight γ fibre running 1 cm. or so parallel to the muscle. In practice this standard is seldom found, and (c) is useful rather in respect of the corollary that if one experimental curve is more foreshortened than another, the fibre corresponding to the first is more oblique to the current than that corresponding to the second.

(*d*) The points whence excitation is elicited are likely to be either where fibres end, or where they bend sharply in such a direction that the current flows into the concavity of the bend.

We may now turn to the experimental results and, using the principles which have been somewhat justified above, we may continue the correlation between γ excitability measurements and the histology of intramuscular nerves.

Analysis of experimental results.

Fig. 9 shows the results of an investigation upon the sartorius of *R. pipiens*. First the muscle was excised, and strength-length curves were obtained in the usual way with the cathode fixed first at 0 mm., then at 40 mm. These curves are shown in the upper part of Fig. 9 where the millimetre scale gives the zero of ordinates. The muscle was then transferred to the rotating trough and the threshold-angle curve obtained using the same duration of stimulus (2σ). These results are shown at the bottom of Fig. 9, plotted as usual in polar coordinates, where O is the polar origin, and where the direction of the muscle is represented by the horizontal broken line. Finally the muscle was fixed and stained according to May's method, mounted in glycerine jelly and traced from an optical projection at magnification of five diameters[1]. The course of the nerves was subsequently verified by microscopic examination of the preparation and comparison with the tracing. This tracing is shown in the middle of Fig. 9.

Turning now to an analysis of the results, it is seen that the strength-length curve with the cathode at the tibial end of the muscle is made up of two parts A and B. The simplest justification for making a kink at 24 mm. in this curve is derived from the curve of reciprocal thresholds (shown below as a curve dipping down to the millimetre scale). This latter curve may be regarded as giving the excitability of the preparation for various positions of the electrodes, and it is seen at once that the point at 24 mm. lies below the smooth curve which might be drawn through the other points. The deviation would correspond to an experimental error of at least 15 p.c. in the threshold, which I do not think is admissible. The curve with the cathode at the pelvic end is obviously composed of three parts, of which C and D are due to the γ excitability, but the remaining

[1] Five diameters is the ratio of the size of the projected image to that of the fresh tissue, as obtained by noting the distances between the principal nerve branches before fixing. In this way errors due to shrinkage during staining are largely reduced.

portion gave an α strength duration curve. This latter portion is a good illustration of the relative independence of the α threshold upon inter-polar length, since there is no further change in threshold after this length has exceeded 3 mm.

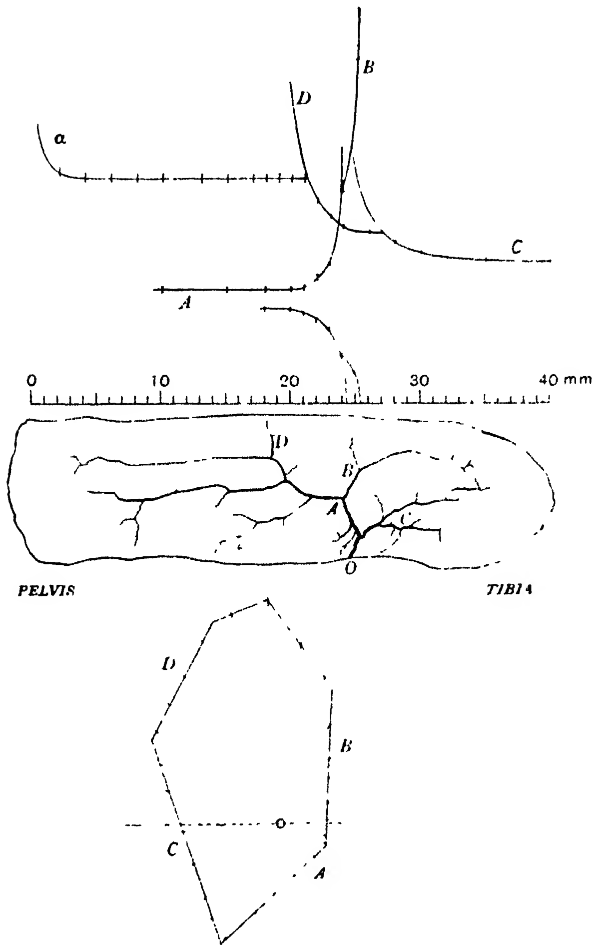

Fig. 9. Strength-length curves, sartorius muscle (*R. pipiens*) drawn to scale, and threshold-angle curve (with coordinate circles omitted for clearness).

Applying now the principles of analysis formulated above we conclude that each of the four curves *A*, *B*, *C*, *D* corresponds to excitation at a different point in the muscle. These points may be found by extrapolating the curves until they become vertical; hence *A* is just to the left

of 25 mm., *B* just to the right of this point, *C* coincides with *A* or lies a little to the left, while *D* is just to the left of 21 mm. Observing now the foreshortening of the curves, it is obvious that *A* and *B* rise very much more steeply than *C* and *D*, therefore the fibres excited in the former case must run much more obliquely to the muscle fibres than in the latter case. These conditions are satisfied approximately if we suppose that curves *A* and *C* represent excitation at *O*, the point of entry of the nerve, the excited fibres in each case being those running from *O* to *A* and *C* respectively in the tracing. It is clear that as predicted branch *AO* is much more oblique than branch *CO*. Curve *B* represents excitation at the point *B* in the tracing, the fibres in question being those running from *A* to *B* and then bending back slightly to the left. Since the current flows into this bend the point is likely to be an excitable one, and the obliquity of both branches should produce a foreshortening of the curve similar to that of curve *A*. Finally curve *D* represents excitation at point *D* in the tracing, the fibres in question running from *A*, bending round *D*, and returning a short way towards the tibia. Curve *D* should be no more foreshortened than curve *C*.

From the foregoing, therefore, we have obtained a definite correlation between the excitation curves and the histology of nerves; it is of interest to compare these conclusions with the threshold-angle results. These results may be all included in six straight lines (as shown), indicating that when the muscle is freely immersed in a uniform field at various angles there are six different points whence excitation is elicited. To identify these we note that the broken line through *O* represents the direction of the muscle, hence the sides of the hexagon cut by this line represent the excitable points when the field is parallel to the muscle. This case, therefore, is the same as that of the strength-length curves above when the interpolar length is very great (*i.e.* the whole muscle exposed). Thus the side of the hexagon marked *C* represents the threshold excitability when the current flows from *O* to the left, which corresponds to the curve *CDα* in the upper figure, which has *C* for the threshold with great interpolar length. But we have identified the excitable point *C* as being *O* on the tracing, the point of entry of those nerve fibres which run in the direction *OC*. From the threshold-angle results, the fibres of the excitability *C* should run in a direction perpendicular to the direction of the side *C* of the hexagon (see p. 180), which is an adequate agreement considering that this simple result is based upon the assumption of a straight fibre.

Applying this analysis to current flowing parallel to the muscle towards the tibia, we learn from the strength-length results that *A* is the

threshold excitability with large interpolar length. This we have identified with O, in the tracing, the point of entry of those nerve fibres which run in the direction OA, and hence the corresponding side of the hexagon should be more or less perpendicular to this direction. It is seen that there is such a side marked A, but this is not the one first cut by the broken line through O. The explanation for the discrepancy is probably as follows. One reason for the presence of the cut nerve end as a principal excitable point in all these experiments is due to the initial hyperexcitability following section. As is well known this excitability declines fairly rapidly, and in the interval between the strength-length and threshold-angle measurements it is probable that the excitability of A and C fell as compared with that of other points not situated at the cut end. Thus the sides A and C of the hexagon moved out from O (keeping the same directions of course), but the other sides remained more or less constant. The lengths of broken line from O to C and from O to A (produced) are in the same proportion as the thresholds for C and A in the strength-length figure, as would be expected. If this explanation is accepted the side A fits the conditions previously postulated for A and we may turn to consider the excitability B.

Excitability B we have supposed to arise from B in the tracing due to the nerve branches lying to the left of this point. When the current excites a nerve at a bend (as distinct from exciting at its termination), the threshold-angle results in polar coordinates give a straight line which is perpendicular to the bisector of the angle of bend in the case where the two limbs are straight and equal. Both theoretical and experimental justification for this statement has been given in a former publication [Rushton, 1928], and applying the results to excitation at B where the assumptions are approximately accurate, we perceive that the bisector of the angle lies in the direction of the muscle, and hence the corresponding side of the hexagon should be perpendicular to this. It is seen that the side marked B fulfils this condition. Excitability D may be analysed similarly, whence we conclude that the corresponding side of the hexagon should be perpendicular to the bisector of the angle at D. The side marked D runs more or less in this direction, and it was recorded when making the observations that the excitability represented by this side gave a barely perceptible twitch, indicating that only a few muscle fibres were activated by the excitability in question, as would be expected if we identify it with the nerve fibres running from D upwards and to the right. The threshold, however, is somewhat higher than might have been expected. With regard to the two upper sides of the hexagon, that on the left gave a very small

twitch and corresponds to some bend of a nerve twig near its termination, but that on the right gave a big contraction and probably corresponds to excitation at A in the tracing, the fibres involved being those which do *not* go in the direction of B, for the bisector of the angle which the remainder make at A is more or less perpendicular to this last side of the hexagon.

This analysis has been considered at some length, not so much for the confirmation which it brings to the γ identification of this paper, as for the analysis itself which, as far as I am aware, has not been previously described and which may have considerable application in various excitability problems. Certain defects in the method are apparent. Localization of electrodes and irregular shrinkage during staining may introduce a possible localization error of $\pm$ 0·5 mm. or even more. The extrapolation of the portions of strength-length curves is somewhat arbitrary, though more reliable when the extrapolation is performed upon the reciprocal curves. Even given approximately the position on the axis of the muscle, where an excitable point is to be located, there may often be several possible branches to choose from, and this possibility will be enormously multiplied if we include those fine terminal nerve twigs which usually do not take the stain in the three hours during which the muscle was generally treated. However, this error is not so great as one might suppose, for it appears to be a general rule that *ceteris paribus* the larger a nerve trunk the more excitable it is, hence the small unstained fibres are not those excited at threshold strengths. However, in spite of this, ambiguity may arise in some cases, which the foreshortening of the strength-length curves and the nature of the threshold-angle curves may help to settle. The threshold-angle curve itself is open to the objection that, since the nerves are not of simple geometrical form, the rules so far applied are more or less inaccurate. Both this and the extrapolation of the strength-length curve are, however, susceptible of more accurate treatment (as it is hoped to show in a future publication); hence the localization can become considerably more determinate than might at first sight appear.

In fact the fourfold correlation—the foreshortening of the strength-length curves, the position of their vertical asymptotes, the nature of the threshold-angle curve, and the histology of nerve distribution in muscle—affords a strong presumption that the basis of the analysis is sound, and that the localizations effected are correct. And when each muscle investigated is found to have a different histology of nerve twigs, a different threshold-angle curve and different numbers and positions of the kinks in the strength-length curves, and yet when these differences are all inter-

correlated in the manner of Fig. 9, there seems little room to doubt that the earlier conclusion of this paper is confirmed in detail and that the γ excitability is to be identified with nerve.

Discussion.

The identification of the γ excitability in muscle is liable to be needlessly complicated owing to the fact that two distinct meanings can be assigned to the expression "γ excitability," and that the identification will depend upon which is adopted. The matter becomes clear from a brief survey of the history.

Lucas [1907–8] showed that when large fluid electrodes are used, α and γ curves may appear, where by γ he meant the curve that he obtained having a short excitation time. Later Jinnaka and Azuma [1922–3], Davis [1922–3] and Watts [1924–5] investigated the effect of size of electrode upon the α excitation time and showed that, as the size of cathode decreased, the α excitation time diminished to about the value which γ had initially. Watts repeated this investigation upon nerve and found that electrode size had a very insignificant effect upon excitation time. With regard to the effect of electrode size upon the excitability which gave a γ curve in Lucas's experiments (large electrodes), these investigators gave little information, as they applied the small cathode almost exclusively to the pelvic end of the sartorius which Lucas had shown to be free from γ.

Upon this head, however, evidence is furnished by the work of Lapicque [1926], who excited the sartorius and other muscles at various points with small cathodes and never found any excitation time different from that nerve. We may, therefore, conclude that γ resembles nerve in that its excitation time is but slightly dependent upon electrode size.

Now, in the conditions of Lucas's experiments, the meaning of "γ excitability" is unambiguous, and the same applies to my own work where the conditions are essentially the same, but when we consider the quickening of the α excitability by the use of small electrodes until the α curve resembles γ, it now becomes merely a question of definition whether we call this altered α curve a γ curve and the responsible excitability a γ excitability or whether we restrict the name "γ" to that excitability which gave γ with large electrodes. In fact, the names α and γ may either be used to describe the behaviour of an excitable element or to distinguish the natures of different elements. It does not matter which definition is adopted, provided the matter is made clear. Actually in this

and in my former papers I have adopted the second alternative, and by γ excitability I mean that element which gives a γ curve when large electrodes are used; the curve obtained by a small electrode from the pelvic end of the sartorius may then unambiguously be described as a γ-like α curve or an α curve of short excitation time.

I much regret that, owing to my failure to be explicit in this matter earlier, Lapicque [1931*a*, p. 202] has evidently supposed my statement that "γ cannot be obtained from the pelvic end of the sartorius" to be a denial of the work of Jinnaka and Azuma and the other workers mentioned above, and has been put to the trouble of repeating their work and confirming that a γ-like curve may be elicited using small electrodes. As a result of these experiments he concludes [1931*b*, p. 219], "I cannot admit that γ corresponds only to the nerve excitability and that the muscle's own excitability corresponds to α," and later, p. 236, "From the above facts, we know that the γ curve is not only concerned with the nervous substance but also with the muscular one." There is no conflict between these statements and the present paper's identification of γ with nerve only, for what Lapicque means (as shown by the nature of his evidence) is merely that muscle can, with stigmatic electrodes, give a γ-like curve. In one of his experiments, however, Lapicque obtains evidence that at first sight does seem to establish that γ may be muscle, for he finds [1931*a*, p. 208] in a curarized muscle without the use of stigmatic electrodes an excitability with rather a short excitation time. The explanation of this case involves a factor of prime importance in the study of α curves, as it may cause the greatest variations in the form of the curve. In my first experiments I found that the α curve varied considerably from one angle to another [1930, Fig. 2]. Subsequent work revealed that the error was due to the irregular terminations of the muscle fibres which were excited by the free immersion of the muscle in fluid. Screening these irregularities from the field allowed of very much more consistent results to be obtained, and I therefore pointed out in my paper that this error was present in the early results but absent from the later ones, and that screening these irregular terminations was essential for consistent results. Unfortunately this observation appears to have been overlooked by Lapicque, for he does not mention it in his criticism of these curves [1931*a*, p. 198], and in his own experiments he has not taken the necessary screening precautions. In fact he writes [1931*a*, p. 199]: "As a first approximation, we considered that the accuracy of parallelism was not necessary, so that I used a round trough," and he proceeds to describe his form of electrodes which not only exposes the ter-

minations of the muscle, but exposes them to an electric field which is anything but uniform. With this arrangement he obtains curves which are seen to vary considerably in shape and to be but poorly reproducible. The question of the variability in the shape of the α curve hardly lies within the scope of the present paper, except in so far as the shape may approach that of the γ curve and be confused with it. This effect I also found in my early experiments when the muscle terminations were not screened, for in experiments with curarized sterno-cutaneous strips sometimes a very brief excitability would be found. The direction of this excitability as given by the threshold-angle relation was always oblique to the muscle, and indeed it was the attempt to identify this oblique excitability that called my attention to the oblique terminations of many muscle fibres. As already mentioned, these γ-like curves did not occur in curarized strips when screened. Moreover, the explanation of the effect is simple, for if we suppose (as Lapicque has already pointed out) that the excitation time of the α curve is greatly diminished by decreasing the interpolar length, then in the case where a parallel fibred muscle is excited by a very oblique current, since the excitable portions of fibre will be those very short terminal irregularities which happen to lie more or less in the direction of the current, they consequently will have the excitation time corresponding to their short length.

Now the case quoted by Lapicque [1931*a*, Fig. 13] where a γ-like curve was found from curarized muscle without a stigmatic electrode is exactly in the category just considered. For not only are the unscreened ends of the muscle exposed to this error, but in comparing the results at the two angles it appears that, whereas the α threshold rises for the oblique current, the γ-like threshold falls, indicating that the direction of the γ-like excitability is oblique to the muscle axis, and therefore certainly cannot refer to normal muscle fibres. It was to avoid the complications produced by these irregularities that at the outset of this paper the conditions were restricted to uniform fields and screened muscle terminations, and in these conditions I do not know of any evidence to controvert the conclusion that γ is always nerve.

Quite a different effect of the muscle terminations upon the excitation time has recently been brought to light by the experiments of Moore and Brücke [1931]. They stimulated the single muscle fibres in the basihyoid membrane of the frog, and found that the excitation time when the cathode lay at the terminations of the fibres depended upon the diameter at this point: thin terminations had long excitation times. They suggested that this might be correlated with α and γ excitabilities in that the γ

might be elicited from the middle of the fibre, and α from the thin end of it. This would fit the observation that in practice α and γ are found more or less in these respective parts of the fibre, and it allows of an isochronism between nerves and the γ excitability of that part of the fibre where the nerve enters. Unfortunately this hypothesis does not accord well with the detailed results of varying the position of the electrodes. When a 1 cm. block is placed over the muscle at various points and the strength duration curve obtained as, for instance [Rushton, 1930, Fig. 8], we do not find that the curve is simple and gradually changing in form from α at the extremity to γ near the centre, but, on the contrary, it is made up of two parts, α and γ, each of which has more or less the same excitation time at all points of the muscle, but the relative prominence of which varies in such a way that γ is absent in the neighbourhood of the extremities, but is present nearly to the exclusion of α in the neighbourhood of the nerve entry. This clearly speaks for two different kinds of excitabilities, not for a continuous variation of one kind, and with regard to α it suggests that it has the same sort of value for its excitation time all over the muscle. With regard to γ, we have the evidence of the present paper which is quite contrary to the suggestion that γ is muscle, since the direction, the action of curare, and all the other features which have been discussed, point strongly to the identification of this excitability with nerve. The suggestion of Moore and Brücke, therefore, will not explain the difference between α and γ, but their observations are valuable in showing that the α excitation time can vary within wide limits depending upon the size of fibre at the point whence excitation is elicited. It is not unlikely, however, that this difference is more marked in the rather heterogeneous fibres of the basihyoid membrane, than in the more uniform fibres of the sartorius.

Conclusions.

This paper set out to identify the γ excitability. Only three excitable elements have been recognized in muscle, the muscle fibre, the nerve fibre and a possible intermediary substance. The latter cannot be identified with γ for the experiments of Fig. 1 show that the γ substance has extension of several millimetres, and consequently must be muscle or nerve. Lapicque [1931, *a*] and Moore and Brücke [1931] have suggested identification with the former, but as was indicated in the foregoing discussion, it is probable that Lapicque's evidence does not relate to what is here called "γ" at all but to a modified form of α, whereas the suggestion of Moore and Brücke is not as satisfactory as the alternative hypothesis

that γ is nerve. This latter view which is that originally advocated by Keith Lucas is very strongly supported by the evidence of the present paper, the conclusions from which may be summarized as follows:

(*a*) The γ excitability has the same excitation time as nerve.

(*b*) The γ excitation time is like that of nerve nearly independent of electrode size.

(*c*) The γ substance is in the form of fibres.

(*d*) In the sartorius these fibres start their course at the exact place where the nerves enter.

(*e*) They run in this muscle towards tibia and towards pelvis for 8 mm. or more.

(*f*) In the sartorius they run in many directions, in the sterno-cutaneous strip they run in the direction of the nerve twig, and more or less perpendicular to the muscle.

(*g*) They are absent from the nerve-free pelvic extremity of the sartorius.

(*h*) When the γ strength-length curves from the sartorius show more than one excitable point, these always correspond to sharp bends in the nerves, and are closely correlated with nerve distribution despite the great variation from preparation to preparation.

(*i*) When the nerve is carefully removed by dissection from the surface of the sartorius, the γ curve initially very prominent disappears entirely from the cleared region.

(*j*) The action of curare upon the γ excitability is to abolish it completely by the time that indirect excitation (through the nerve) has failed.

As a result of this summary there can be no question I think, but that the γ fibres in all my experiments were the intramuscular nerve twigs. With regard to the possiblity that in the foregoing experiments it so happened that the nerves and the α excitability between them were always more excitable than some hypothetical γ muscle fibres, so that the latter were never investigated, little need be said. If these hypothetical fibres never enter into threshold measurements we are justified in neglecting them when concerned with threshold determinations, and it will be time enough to consider their nature when some reason is found for supposing they exist. To quote from Lapicque [1926, p. 265], "Était-il besoin d'entreprendre la démonstration complète? Une démonstration négative est toujours bien laborieuse; la preuve incombe à ceux qui affirment."

Summary.

When a muscle is excited through large fluid electrodes, two excitabilities α and γ are found. The present paper set out to identify γ in conditions where the irregular terminations of the muscle are not excited and where the stimulating electric field is uniform. In these circumstances a variety of experiments with movable electrodes justify the ten conclusions summarized in the preceding section, whence it appears that the γ excitability is certainly nerve.

It is a pleasure to express my appreciation of the help afforded me by Prof. Bronk during my stay in Philadelphia. I am also indebted to the Government Grants Committee of the Royal Society for enabling me to obtain some of the apparatus used in this research.

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THE EFFECT OF THE INJECTION OF BLOOD ON THE UREA IN BLOOD AND URINE.

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Every transfusion of blood is an attempt at transplantation of living tissue, and involves the introduction parenterally of a considerable quantity of protein. According to investigations of Carlson and Ginsburg [1915] and of Rabens [1915], after transfusion of compatible blood into an animal there is no marked change in the excretory mechanism of the kidney. In cases of the failure of blood transfusion, where the bloods are incompatible, a range of symptoms of varying severity may be observed, including hæmoglobinuria. If the protein injected is broken down in the blood stream and tissues by proteolytic enzymes, there must be an increase in some products of protein breakdown: proteoses and amino-acids, the deaminization of the amino-acids liberating additional quantities of urea. Whipple and van Slyke [1918] and Kambe Hisanobu [1919] have shown that some intermediate products of protein breakdown, when present in the blood, affect the kidneys, interfering with their function either directly, or indirectly by some alteration in the circulation.

The question as to the influence of incompatible blood on the function of the kidney cannot be considered as solved by physiological experiments up to the present. Nevertheless in recent years we see an attempt to revive the old idea of transfusion of blood of certain animals into the human body [Cruchet, 1926, 1928; Yourewitch and Teleguina, 1925; Kunz, 1928, 1929]. This suggestion has been much criticized by many scientific workers as ignoring the recent data on the incompatibility of blood. These considerations led to the present study of the influence of blood transfusion on the concentration of urea in blood and the elimination of urea by the kidney.

These experiments were made upon twelve well-nourished dogs (6·0–9·5 kg.). In each animal a permanent bladder fistula was made about a fortnight before it was used for experiment. To exclude any influence

of processes of digestion on the urea level in blood and urine our observations were made on dogs to which no food had been given for 12 hours before the experiment, although water was freely available both before and after the blood transfusion.

The experiments performed can be divided into five series:

(1) Observations upon the blood urea and urine urea in unfed dogs: 4 experiments.

(2) Effect of transfusion of dog's blood into dogs: 2 experiments.

(3) Effect of transfusion of incompatible blood into dogs: 5 experiments.

(4) Effect of successive transfusion of citrated plasma and washed blood corpuscles: 4 experiments.

(5) Effect of repeated transfusion of incompatible blood: 5 experiments.

In addition one experiment was made to study the effect of tying the ureters on the blood urea in the dog. The increase in blood urea obtained thus could be compared with the increase observed in experiments of series 3–5.

Method of experimentation.

Under ether anæsthesia, blood was collected from the carotid artery in dogs and the abdominal aorta in cats and rabbits into a glass receiver containing 10 p.c. sodium citrate (5 c.c./100 c.c. blood) to prevent coagulation. For the separate transfusion of plasma and blood corpuscles, the blood was centrifugalized, the plasma pipetted off and the corpuscles, after washing twice on the centrifuge with sterile saline, were suspended in saline made up to the original volume of the blood. This suspension was kept for transfusion till the next day in cold storage. The whole blood, plasma and washed blood corpuscles were warmed to 38° C. before transfusion.

Under ether anæsthesia, preceded generally by morphine, the blood was injected into the vein from a burette kept at 38° C. The rate of transfusion during the first 2–3 min. did not exceed 1 c.c. per min.; afterwards it was gradually increased to 5 c.c. per min. After transfusion the central end of the vein was ligated and the wound was closed with silk. A collodion dressing was used. The whole operation was carried out with aseptic precautions.

Immediately after the transfusion the bladder of the dog was emptied through the cannula. Most of the dogs had recovered from the immediate effect of the operation within 30 min. from the end of transfusion, and

appeared normal. About 4 c.c. of blood for analysis were collected from the veins of the ear in a vessel containing a small quantity of potassium oxalate. Samples of blood and urine were always taken before transfusion, and subsequently at intervals usually of 1 or 2 hours for the rest of the day and at longer intervals afterwards. If the determination of urea was postponed the test-tubes containing the blood or urine were placed in the ice-box, toluene having been added to the urine. The analyses were made after the samples had been warmed to room temperature.

The estimations of the urea in blood and urine were made by van Slyke's method (aeration method) as described by S. W. Cole [1928]. In urine the ammonia was estimated separately and deducted.

A. *Determination of the blood urea, urine urea and urea output in fasting dogs.*

In four dogs, determinations made six or eight times during the day showed that the concentration of urea in the blood remained almost or quite constant, while the concentration of urea in the urine showed marked variations (Table I). In one dog in which the volume of urine per hour was measured, the hourly output of urea from the body was fairly constant.

TABLE I

	Blood urea (mg./100 c.c.)	Urine urea (g./100 c.c.)	Urea output (g. per hr.)
Dog 1	32·8	1·2–3·2	
„ 2	32·0	3·6–4·5	
„ 3	28·0	1·2–4·6	
„ 4	22–25·8	2·35–3·95	0·202–0·257

B. *Transfusion of dog's blood into dogs.*

Dogs 1 and 3 received 10 c.c./kg. of dog's blood with no ill effects and without any change in the concentration of urea in the blood during the subsequent 8 or 9 hours, nor was this changed on the following day.

C. *Transfusion of incompatible blood into dogs.*

In all transfusions of incompatible blood the quantity of urea was determined in the blood of the donor. This ranged from 20 to 28 mg./100 c.c. for cat's blood, and from 16·5 to 24·75 mg. for rabbit's blood.

Two dogs received intravenous injections of cat's blood. Both animals survived and were used for subsequent experiments. A summary of the results obtained is given in Table II.

In these dogs, unlike those which received dog's blood, the urea in the blood was increased to a maximum about the sixth hour, in each case

TABLE II.

Dog. 5. Weight 8 kg. Transfusion of 80 c.c. cat's blood.
Dog 2. Weight 7 kg. Transfusion of 70 c.c. cat's blood.

Dog 5.

Date	Time of transfusion	Hour	Urea in Blood mg. p.c.	Urea in Urine g. p.c.	Urine flow c.c. per hour	Urea elimination g. per hour
27. ii. 31	11.40–	10 a.m.	42·0	4·3	—	—
	11.52 a.m.	11 a.m.	42·0	4·8	7·2	0·325
		12.52 p.m.	46·7	3·0	7·0	0·201
		1.52 p.m.	51·38	1·84	14·0	0·258
		2.52 p.m.	51·5	2·0	13·0	0·260
		3.52 p.m.	56·0	2·0	10·0	0·20
		5 p.m.	65·5	2·3	8·5	0·195
		6 p.m.	65·5	2·0	14·5	0·29
		7 p.m.	65·5	2·3	13·0	0·30
		9 p.m.	65·5	2·7	21·0	0·567
28. ii. 31		9.30 a.m.	46·7	4·8	—	—
		6 p.m.	42·0	4·1	—	—
		Dog 2.				
6. iii. 31	10.15–	8.40 a.m.	28·1	—	—	—
	10.27 a.m.	9.40 a.m.	28·1	1·0	—	—
		11.30 a.m.	46·7	1·28	17·0	0·218
		12.30 p.m.	46·7	1·13	35·0	0·395
		1.30 p.m.	46·7	2·0	10·0	0·2
		2.30 p.m.	46·7	2·3	6·0	0·136
		4.30 p.m.	46·7	2·0	22·0	0·44
		5.30 p.m.	51·4	1·9	8·0	0·152
		6.30 p.m.	51·4	2·1	7·0	0·147
		7 30 p.m.	46·7	2·5	6·0	0·15
7. iii. 31		10 a.m.	46·7	—	—	—
		6 p.m.	28·0	—	—	—

by 23 mg./100 c.c. more than before transfusion. It was still a little up next morning but fell by evening. The concentration of urea in the urine fell in dog 5 from a high figure to about 2 p.c., and in dog 2 rose from a low figure to about 2 p.c. There was no evidence of damage to the kidney in either animal, and there was no hæmaturia.

Rabbit's blood was transfused into three dogs (6, 7 and 8). Marked signs of depression in varying degree were observed as may be seen from the following condensed protocols, referring to dogs 6 and 8. The results of the experiments are given in Table III. Dog 7 gave results similar to dog 6.

Dog 6.

11.15–11.27 a.m. Transfusion of 35 c c. (5 c.c./kg.).
12.30 p.m. Dog drank 50 c.c. of water.
12.30–4.30 p.m. Dog vomiting. Urine contained blood.
5.15 p.m. Dog drank 50 c.c. of water
5.30–8.30 p.m. Urine dark with altered blood.

Dog 8.

11.55 a.m.–12.5 p.m. Transfusion of 35 c.c. (4 c.c./kg.).

1.5 and 2.5 p.m. Very marked depression. Unable to stand. The legs and ears cold. In half an hour was possible to collect from the ear only 2·0 c.c. of blood. No urine.

2.50 p.m. Vomiting. Dog was standing up.

3.5 p.m. Condition became rapidly worse, with marked loss of sensitiveness.

7.10 p.m. Vomiting.

7.30 p.m. The dog died.

TABLE III.

Dog 6. Weight 7·0 kg. Transfusion of 35 c.c. rabbit's blood.
Dog 8. Weight 9·0 kg. Transfusion of 35 c.c. rabbit's blood.

Dog 6.

Date	Time of transfusion	Hour	Urea: Blood mg. p.c.	Urea: Urine g. p.c.	Urine flow c.c. per hour	Urea elimination g. per hour
21. iv. 31	11.15–	10 a.m.	16·5	—	—	—
	11.27 a.m.	11 a.m.	16·5	2·93	4·2	0·12
		12.30 p.m.	28·9	0·65	0·7	0·005
		1.30 p.m.	37·2	0·66	3·0	0·02
		2.30 p.m.	41·2	1·11	3·2	0·04
		3.30 p.m.	53·6	1·22	1·5	0·02
		4.30 p.m.	53·6	1·57	3·0	0·047
		5.30 p.m.	53·6	1·38	8·0	0·11
		6.30 p.m.	49·5	0·8	40·0	0·32
		7.30 p.m.	41·2	1·1	32·0	0·35
		8.30 p.m.	41·2	1·24	30·0	0·37

Dog 8.

Date	Time of transfusion	Hour	Urea: Blood mg. p.c.	Urea: Urine g. p.c.	Urine flow c.c. per hour	Urea elimination g. per hour
25. iv. 31	11.55 a.m.–	10.25 a.m.	24·75	3·1	2·5	
	12.5 p.m.	11.25 a.m.	24·75	—	2·2	
		2.5 p.m.	33·0	—	0	
		4.5 p.m.	45·4	—	0	

Dog died at 7.30 p.m.

In dog 6 there is a maximal increase in the quantity of the blood urea to 53 mg. by the fourth hour after transfusion. The blood urea remains at that level for nearly 4 hours, and then decreases. For 5 hours after transfusion there is a decrease in the urea concentration without any increase in the quantity of urine eliminated per hour. The decrease in the ability of the kidney to concentrate urea is observed during the whole time of the experiment. The hourly output of urea is thus diminished during the 5 hours following blood transfusion. After drinking 50 c.c. of water, the urine flow rapidly increases, with increased urea elimination and a fall in blood urea.

Certain deductions may be based on the experiments of this series. Following transfusion of incompatible blood into dogs (from cat and rabbit):

(*a*) Sometimes there is a fall in the volume of urine during the first

hour after blood transfusion, and normal or even copious elimination of urine in subsequent hours.

(b) Rabbit's blood has a more pronounced toxic action than cat's blood. Although smaller amounts of rabbit's blood were used, the increase in blood urea was more marked, and in the experiment with dog 8 a complete suppression of urine and the rapid death of the animal resulted.

D. *The effect of successive transfusion of citrated plasma and of washed blood corpuscles.*

Experiments were made to ascertain whether the effect of the transfusion of blood might be ascribed to the whole tissue or whether there was some difference in the action of the chief constituents of blood.

Table IV shows the results of three experiments, in which transfusion on the first day of 50 c.c. of citrated cat's plasma was followed on the second day by transfusion of a suspension in 70 c.c. of saline solution of the washed corpuscles of the same cat's blood (100 c.c.).

TABLE IV. Effect of successive transfusion of cat's plasma and corpuscles on blood urea in mg./100 c.c.

	Dog 9 (7 kg.)		Dog 4 (8 kg.)		Dog 7 (8 kg.)	
	Plasma	Corpuscles	Plasma	Corpuscles	Plasma	Corpuscles
Initial value	16·5	16·5	24·3	28·0	20·7	20·7
Hours after transfusion						
1	16·5	16·5	28·0	46 2	35·3	20·7
2	24·8	24·8	35·3	53·5	35 3	28·0
3	24·8	24·8	35·3	53·5	—	—
4	16·5	24·8	—	57 2	35·3	38·9
5	16·5	24·8	28·0	—	31·6	42·6
6	16·5	28·9	24·3	57 2	31·6	42·6
7	16·5	33·0	24·3	—	28·0	38·9
8	16·5	24 8	28·0	53·5	28·0	—
9	16·5	24·8	24·3	57·2	—	—

After plasma transfusion, the increase of blood urea lasted for 4 hours at most, while after transfusion of corpuscles the increase of blood urea was more prolonged and in two of the experiments was much greater. But with the corpuscles, a much larger dose of foreign protein was injected than with the plasma transfusion.

The results of one experiment in which the plasma and corpuscles of rabbit's blood were transfused are given in Table V. After plasma transfusion there were no objective symptoms. Three hours after the transfusion, the dog drank 50 c.c. of water. Three-quarters of an hour after the transfusion of corpuscles, vomiting occurred, and during the next

Table V. Dog 10. Weight 6·5 k.g. Transfusion of (i) 30 c.c. citrated plasma and (ii) washed corpuscles of the same rabbit's blood (50 c.c.)—suspension in 50 c.c. of saline solution.

Date	Time of transfusion	Hour	Urea: Blood mg. p.c.	Urea: Urine g. p.c.	Urine flow c.c. per hour	Urea elimination g. per hour
		Plasma transfusion.				
22. iv. 31	12–	10.30 a.m.	16·5	3·74	—	—
	12.8 p.m.	11.30 a.m.	16·5	4·26	2·7	0·115
		1.10 p.m.	24·8	1·7	5·0	0·085
		2.10 p.m.	24·8	1·8	6·0	0·105
		3.10 p.m.	24·8	3·7	2·0	0·074
		4.10 p.m.	24·8	1·88	6·5	0·122
		5.10 p.m.	20·6	2·67	5·0	0·133
		6.10 p.m.	20·6	2·65	3·5	0·093
		7.10 p.m.	20·6	2·35	3·0	0·07
		8.10 p.m.	16·5	4·1	3·0	0·123
		9.10 p.m.	24·8	4·48	2·0	0·09
		Blood corpuscles transfusion.				
23. iv. 31	10.56–	9.30 a.m.	24·8	4·06	—	—
	11.8 a.m.	10.30 a.m.	24·8	4·23	9·0	0·386
		12.10 p.m.	37·1	1·91	5·0	0·096
		1.10 p.m.	37·1	1·8	6·5	0·117
		2.10 p.m.	37·1	1·9	5·0	0·097
		3.10 p.m.	37·1	1·8	8·5	0·15
		4.10 p.m.	33·0	0·39	57·0	0·222
		5.10 p.m.	37·1	0·39	57·0	0·222
		6.10 p.m.	45·4	0·92	22·0	0·203
		7.10 p.m.	45·4	1·68	11·0	0·184
		8.10 p.m.	49·5	2·12	9·5	0·202
24. iv. 31		3.5 p.m.	33·0	—	7·5	
					10·6	
					9·0	

$2\frac{1}{4}$ hours the dog drank 150 c.c. of water. As with cat's blood, the rabbit's plasma transfusion caused a less marked rise in blood urea than did transfusion of rabbit's corpuscles. The hourly output of urea was markedly decreased for 9 hours after corpuscle transfusion. There was absence of hæmaturia which occurred in the two dogs which survived the injection of 35 c.c. of whole rabbit's blood.

E. *Repeated transfusions of incompatible blood into dogs.*

According to Friedberger (quoted by Vigano), anaphylaxis can be produced with great facility in dogs which are quite as sensitive as guinea-pigs. Vigano [1928] points out that sometimes the only symptom of anaphylaxis in dogs is a lowering of the blood-pressure.

Repeated transfusions of incompatible blood are interesting from two points of view. Firstly, with anaphylaxis, phenomena met with during the first transfusion were expected to occur in an accentuated state and secondly, as several authors observed during the anaphylaxis a pro-

nounced increase of blood urea, the intention was to compare the changes in the quantity of urea in blood with those in the urine. This might throw light upon the true participation of the kidney in some reactions following transfusion of incompatible blood.

Below are given the results which followed a second transfusion of 50–75 c.c. of cat's blood made into three dogs which had each received an initial transfusion 6–9 weeks earlier.

Dog 12. Hæmaturia starting within 1 hour of transfusion and continuing.

The percentage of urea in urine and the total output of urea fell considerably and remained at very low values.

By the ninth hour after transfusion the blood urea had risen to 70 mg./100 c.c. from an initial value of 28 mg., and at death, 3 days after transfusion, it was 250 mg.

Dog 2. Hæmaturia within 1 hour of transfusion.

Almost complete suppression of urine.

By seventh hour after transfusion (just before death) the blood urea had risen to 93 mg./100 c.c. from an initial value of 35 mg.

Dog 11. Complete suppression of urine.

At death, 6 hours after transfusion, blood urea had risen to 49 mg./100 c.c. from an initial value of 20 mg.

In two dogs the transfusion of rabbit's blood was repeated with the following results:

Dog 6 (7 kg.). Initial transfusion 35 c.c. Second transfusion (5½ weeks later) 40 c.c.

Repeated vomiting.

Hæmaturia, with marked fall in urine flow and urea output.

Blood urea rose from initial value of 22 mg./100 c.c. to 262 mg. at death during second day after transfusion.

Dog 10 (6·5 kg.). Initial transfusion 30 c.c. Second transfusion (6 weeks later) 50 c.c.

Repeated vomiting.

Hæmaturia—urine free from blood 24 hours after transfusion.

The urea output diminished soon after transfusion, and was increased during the diuresis occurring after copious drinking; at the same time, the blood urea which had risen from an initial value of 24 mg./100 c.c. to 73 mg. began to decrease, and by the third day was down to the initial value.

The recovery of dog 10 from the anaphylactic renal poisoning is remarkable and probably resulted from the copious drinking with consequent flushing of the renal tubules.

F. *The effect of tying the ureters on the blood urea.*

One experiment was performed to determine the effect on the blood urea of the gradual cessation of urine formation resulting from ligature of both ureters. Within 1 hour of ligature, the blood urea had risen from the initial value of 24 mg./100 c.c. to 35 mg. Eleven hours later it was 177 mg., and 31 hours after ligature it had reached 203 mg.

Discussion of results.

The objective symptoms of incompatibility of bloods in these experiments were, besides agglutination and hæmolysis, the appearance in the plasma of the recipient of dissolved hæmoglobin and its excretion in the urine. In all the cases of transfusion into dogs of cats' and rabbits' blood we observed that the recipient's blood plasma was tinted with hæmoglobin. Also following all heterogeneous transfusions where the kidney was active, there was hæmaturia starting $\frac{1}{4}$–$\frac{1}{2}$ hour after transfusion and usually continuing for 8–10 hours. Where compatible blood was transfused (dog's blood into the dog), the above-mentioned symptoms were, of course, absent.

The increased quantity of blood urea following the transfusion of the heterogeneous blood should be an index of the degree of decomposition of the injected proteins. It is known that parenterally introduced protein can be broken down in the body into proteoses, polypeptides and amino-acids which are either utilized by the tissues or undergo deaminization, giving rise to an additional quantity of urea. This urea must be eliminated from the organism by the kidney.

Determinations of the blood urea in unfed dogs have shown the absence of any pronounced variation during 1 day, as has also been shown for man by Daumas and Pagès [1930]. Moreover, the experiment on dog 4 showed that the quantity of urea eliminated under these conditions is nearly constant over a period of 7 hours. The only variations were in the percentage of urea in urine and in the quantity of urine eliminated per hour.

The question arises, whether the increase of blood urea found after transfusion of incompatible blood depends entirely on the breakdown of the injected proteins or whether it does not also result from a temporary failure of the kidney to turn out urea, due either to vascular changes or to a poisoning of the renal cells. Mioni [1906], Studzinski [1909], Bayliss [1920] and others have noticed a fall of blood-pressure in animals following the transfusion of the heterogeneous blood. This they explained as due to the action of some product of the breakdown of proteins in the blood stream (proteose), as it occurred during anaphylactic shock. But experiments of Mioni, confirmed by the present work, have shown that the prompt fall in pressure after the transfusion of incompatible blood is only transient. Recently Mason and Mann [1931] have found that contraction of the renal vessels follows intravenous injection of laked blood into dogs, but the effect is only transitory. Inspection of Tables II and

III shows that any diminution of urine flow was temporary (except in the case of dog 8, where there was complete suppression of urine). From the second hour at latest after blood transfusion there was a normal or even an increased urine flow. This shows that the increase in blood urea cannot be explained by an insufficient elimination by the kidney dependent on vascular changes. These experiments show quite clearly that there is a definite insufficiency of the kidney itself following transfusion of the heterogeneous blood. Thus, in all experiments except one, we have noticed a decrease in the percentage of urea in urine for several hours after the transfusion. This decrease of the capacity of the kidney to concentrate urea can only be explained by specific action of some definite products of breakdown of the heterogeneous blood in the blood stream of the recipient. We find a decrease, sometimes very pronounced, in the hourly output of urea. We also find in many cases that the increased diuresis (the compensatory mechanism) for several hours after the blood transfusion could not restore the normal elimination of urea. Our assumption that the temporary accumulation of blood urea following the heterogeneous blood transfusion must be explained by a temporary intoxication of the kidney by some products of breakdown of the injected blood seems to us to be proved also by the experiments with repeated transfusion of incompatible blood. The difference between the effects of the first and second transfusion of blood is quantitative rather than qualitative. It is true that in the cases of anaphylactic shock we sometimes meet with complete suppression of urine, but in some experiments (dogs 12 and 10) of this series the urine flow was either to some extent quickly restored, or even exceeded the pre-transfusion value. Repeated transfusion was followed not only by a rapid increase in the quantity of blood urea but also by a sharp decrease in the concentration of urine urea and a diminution in the quantity of urine eliminated hourly, due to renal poisoning.

Mioni [1906] showed that intravenous injection of heterogeneous erythrocytes into dogs caused vascular changes similar to those following proteose injections, while heterogeneous serum caused weaker and more transient changes. De Kruif [1917] and Drinker and Brittingham [1919] showed that homogeneous plasma is singularly non-toxic as compared with serum. It is to be expected, therefore, that the effect of heterogeneous corpuscles on the kidney will be greater than that due to heterogeneous plasma. The present experiments indicate this, but it is necessary to bear in mind that the amount of foreign protein injected was much greater in the corpuscle transfusion than in the transfusion of plasma.

Conclusion.

1. In the normal unfed dog: (*a*) the blood urea level is constant or nearly constant over a period of 6–10 hours; (*b*) the quantity of the urea eliminated per hour by the kidney is almost constant.

2. Following transfusion of dog's blood into dogs: (*a*) the blood urea level is not affected; (*b*) the curve of urine urea has its usual character.

3. Transfusion of cat's blood into dogs results in an increase of blood urea which reaches its maximum after 4–6 hours. The normal concentration is restored within 24 hours.

4. Transfusion of rabbit's blood into three dogs results in severe damage to the kidneys, with fatal effects in one dog.

5. Transfusion of washed blood corpuscles causes a more pronounced increase in the quantity of blood urea than that following the transfusion of the citrated plasma of the same blood. This may be due, at any rate in part, to the larger dose of protein given in the corpuscle transfusion.

6. Repeated transfusions of incompatible blood (from cat or rabbit into dog) result in acute anaphylactic renal poisoning. It is remarkable that one out of the five animals recovered.

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THE LACTIC ACID METABOLISM OF FROG'S MUSCLE POISONED WITH IODOACETIC ACID.

I. The lactic acid metabolism of anaerobic iodoacetate muscle.

II. The lactic acid metabolism of aerobic iodoacetate muscle.

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I. THE LACTIC ACID METABOLISM OF ANAEROBIC IODOACETATE MUSCLE.

THE work of Lundsgaard [1930] has demonstrated that a muscle poisoned with iodoacetic acid can do a considerable amount of work without any production of lactic acid. The source of chemical energy for both normal and poisoned muscles is to be found in the heat of reaction of the breakdown of creatinephosphoric acid to creatine and phosphoric acid, and in a normal muscle the energy necessary to allow of the resynthesis of creatine phosphoric acid is produced, at any rate in the main, by the breakdown of glycogen to lactic acid. Finally, the *status quo ante* is restored by the resynthesis of glycogen from four-fifths of the lactic acid produced, the necessary energy being provided by the combustion of the remaining one-fifth of the lactic acid. Such, in brief, is believed to be the course of the main events during and after a muscular contraction.

In a poisoned muscle, however, the production of lactic acid is inhibited, and although glycogen breaks down it is converted to hexose mono- and diphosphoric esters [Lundsgaard, 1930] with very little production of energy, so that the restoration of the creatinephosphoric acid broken down during activity is rendered impossible. When, therefore, the store of this essential material becomes exhausted, the muscle ceases to twitch and goes into a peculiar type of rigor. This phenomenon occurs under both aerobic and anaerobic conditions, although an oxygenated muscle will usually survive longer than an anaerobic muscle [Lundsgaard, 1930].

It might be that in iodoacetate muscle lactic acid is actually produced, but is subsequently removed at a sufficiently rapid rate to prevent its detection, and previously published evidence does not seem to preclude this possibility. In a form of poisoning closely resembling that of iodoacetate, however, it has been shown [Lipmann, 1927] that lactate is not removed when added to minced anaerobic muscle in the presence of fluoride, but it seemed profitable to carry out a series of experiments to investigate the possibility of removal of lactate by muscle in the presence of iodoacetate.

In the first instance, minced frog muscle was incubated for a sufficient time in presence of prussic acid to ensure the production of a considerable amount of lactic acid. Potassium iodoacetate was then added to one portion and incubation continued. No appreciable reduction in the amount of lactic acid present could be obtained by this procedure.

An attempt was also made to obtain removal of lactic acid from lactate solution added to freshly minced muscle, but neither incubation of the material with iodoacetate nor leaving the same mixture at room temperature for a considerable period caused any reduction in the amount of added lactate.

Experiments on similar lines were then carried out on intact frogs. The animals were pithed and the feet pierced with silver wire electrodes. After giving 100 "make and break" maximal shocks the heart was exposed, the sinus venosus punctured, and 5 c.c. of iodoacetate solution in phosphate-Ringer perfused slowly through the frog by injection of the fluid into the aorta. Immediately after this treatment one leg was cut off and worked up for lactic acid, and the other leg allowed to go into rigor. This usually took place after 45 to 100 minutes. The remaining leg was then treated in exactly the same manner as the leg which had previously been cut off.

It was considered probable that, if anaerobic reduction of lactic acid did occur at all, the leg which had gone into rigor would have contained little or no lactic acid, but this was not found to be the case. The perfusion of the frog must necessarily have washed out considerable amounts of pre-formed lactic acid, but in general similar amounts would be lost from either leg, and it was believed at first that evidence had been obtained of a reduction in lactic acid content in the "rigor" leg of the order of 25 p.c. Control experiments, however, showed that this apparent reduction was due to the fact that iodoacetate poisoning is a time reaction [Lundsgaard, 1930].

During the mincing of the "cut off" limb lactic acid was produced,

because it was worked up immediately after the perfusion, whereas the "rigor" limb gave rise to no lactic acid during the mincing period. The difference in lactic acid content of the two limbs, in fact, disappeared if the "cut off" limb was removed 10–17 minutes after the end of the perfusion and the "rigor" limb after a further hour's contact with iodoacetate.

The conclusion, then, can be drawn that neither in minced muscle, nor in muscle in the intact limb of the frog, can lactic acid be removed anaerobically in the presence of iodoacetate.

Experimental.

A series of experiments was carried out in which muscle from four winter frogs was minced in a mincer cooled in ice, and the pulp divided into three portions in a cold dish. One portion was worked up for resting content of lactate, one incubated at 37° C. for 90 minutes with two drops of HCN and 2 c.c. of $N/100$ potassium iodoacetate solution. The third portion was treated in a similar way, but without addition of iodoacetate. The results (Table I) show that the muscle incubated in presence of iodoacetate produces no appreciable quantity of lactic acid. The percentages of lactic acid are calculated on moist muscle weight.

Table I.

Lactic acid (p.c.)

Exp. No.		Resting muscle	Incubated with iodoacetate	Incubated alone
17		0·082	0·095	0·329
20		0·052	0·038	0·236
23		0·064	0·096	0·374
30		0·064	0·068	0·253
	Mean	0·066	0·069	0·298

In the next series of experiments the muscle was minced and incubated at 37° C. for $2\frac{1}{2}$ hours. It was then divided into three portions, one of which was incubated for a further 3 hours with addition of two drops of HCN. The second portion was incubated for a similar period with 2 c.c. of $N/100$ potassium iodoacetate and a similar amount of HCN, and the third portion was treated in the same way, but incubated overnight. The results given in Table II show that no appreciable reduction of lactic acid took place in the presence of iodoacetate.

Lithium lactate solution was then added to freshly minced muscle, as it was thought possible that the enzyme system might have been disturbed by previous incubation of the pulp. It is known, for instance

TABLE II.

Exp. No.	Lactic acid (p.c.) Incubated 3 hr.	Incubated 3 hr. with iodoacetate	Incubated overnight with iodoacetate
24	0·457	0·451	0·496
27	0·516	0·490	0·500
30 A	0·253	—	0·220

[Lohmann, 1926], that incubation of muscle extract for 15 minutes at 37° C. will destroy the glycolytic activity of the extract.

The minced muscle was divided into four portions. One was estimated as resting muscle. The next was treated with 1 c.c. of lactate solution and also given resting muscle treatment, the difference between the two giving the amount of added lactate. The same amount of lactate was added to the third portion, which was incubated for 4 hours with iodoacetate. The last portion was incubated alone for 4 hours. Toluene was used as a preservative in each case. In column 4 the added lactate has been subtracted from the observed lactate.

TABLE III.

Exp. No.	Lactic acid (p.c.) Resting	Resting + Lactate	Incubated 4 hr. + Lactate and iodoacetate (less added lactate)	Incubated 4 hr.
40	0·041	0·238	0·032	0·286
41	0·064	0·244	0·063	0·300
44	0·048	0·217	0·042	0·289

In column 4 it is shown that the difference between the observed lactate and added lactate is a close approximation to the value for the resting muscle, as would be expected from the results given in Table I had no disappearance of lactate taken place. If any lactate had disappeared, subtraction of "added" from "observed" lactate would have given negative values in column 4.

In the experiments on intact frogs the perfusing Ringer solution contained $N/2000$ potassium iodoacetate (1 part in 10,000). The amount of lactic acid found respectively in "cut off" and "rigor" limbs is given in Table IV. The amounts are expressed as percentages of the dry weight of the muscles as the perfusion gave rise to an œdematous condition in some cases.

TABLE IV.

Exp. No.		Lactic acid (p.c.) "Cut off" limb	"Rigor" limb
59		0·434	0·366
65		0·621	0·474
67 A		0·332	0·208
67 B		0·384	0·244
61		0·691	0·535
	Mean	0·492	0·365

The difference of 0·127 p.c. appeared significant, but some doubt arose when a control experiment was done using phosphate-Ringer solution without addition of iodoacetate. This gave 0·252 p.c. lactic acid in the limb immediately severed and only 0·111 p.c. in the limb left for an hour, but a more important result was obtained by perfusing four frogs with Ringer containing $N/100$ iodoacetate and estimating lactic acid in their muscles, removed and minced in a warm room without precautions after different periods of rest.

TABLE V.

Time of severance after perfusion (min.)	0	5	15	30
Lactic acid on dry weight (p.c.)	0·585	0·495	0·221	0·137

The "injury" production of lactic acid is clearly likely to be much greater in the "cut off" limb than in the "rigor" limb, where it will be negligible. This point was proved by experiment on frogs perfused with $N/300$ iodoacetate Ringer solution.

TABLE VI.

Exp. No.		Lactic acid (p.c.) Cut off	Left 17 min.
56 A		0·382	0·191
56 B		0·291	0·241
57		0·149	0·084
58		0·173	0·116
	Mean	0·249	0·158

The mean difference of 0·091 p.c. is clearly of the same order as that of 0·127 p.c. in Table IV. The point was finally settled by doing experiments in which, in each experiment, two frogs were used. One was treated as before, one leg being cut off immediately after perfusion, and the other when rigor had set in. The other frog was allowed to remain 10–17 minutes after perfusion before the "cut off" leg was removed. Two typical results are given in Table VII.

TABLE VII.

Exp. No.	Iodoacetic acid concentration	No. of frog	Time of rest (min.)	Lactic acid (p.c.) Cut off	Rigor	Difference
54	$N/100$	1	0	0·590	0·470	0·120
		2	17	0·440	0·435	0·005
55	$N/300$	1 A	0	0·500	0·232	0·268
		2 A	10	0·295	0·270	0·025

It is therefore clear that, if the muscle is allowed to remain until it is thoroughly poisoned, no evidence of reduction of lactic acid content in presence of iodoacetate can be obtained by perfusion of the intact frog.

II. THE LACTIC ACID METABOLISM OF AEROBIC IODOACETATE MUSCLE.

Since the earliest work by Lundsgaard [1930] on the metabolism of muscle poisoned with iodoacetic acid it has become increasingly clear that the alteration in the chemical mechanism of the contractile process is not to be looked for in the earlier stages of the contraction-relaxation cycle of the stimulated muscle. Myothermic work by Hartree [1931] and Fischer [1931] demonstrated that not only was the appearance of the heat-production curve the same in poisoned as in normal muscle, but that the relationship between tension developed and heat produced was also identical in the two cases. The identity of the processes during the early stages of contraction of normal and poisoned muscles was shown to be possible when Lehnartz [1931] demonstrated the truth of the contention that most of the lactic acid was produced after the conclusion of the mechanical response. The mechanical response itself was investigated by Henriques and Lundsgaard [1931], who found that the latent period, period of contraction, and course of the contraction curve as well as the tension-length developed, were quite normal in iodoacetic acid muscle.

From these results it is clear that, in the earlier stages of a response to stimulation, a muscle poisoned with iodoacetic acid behaves as if it were a normal muscle. If, therefore, we are to find the link in the chain of events which is displaced by the action of the poison we must investigate the later stages of the response, and in this respect observation of the respiration of poisoned muscle assumes particular interest. Meyerhof and Boyland [1931] showed that the respiratory quotient of poisoned muscle was in the neighbourhood of 0·7, which would seem to indicate that the fuel being utilized was fat rather than carbohydrate. Addition of lactate to the muscle restored the respiratory quotient to a value of 0·95, which

is that observed for normal muscle, but the most striking feature of the observations was the threefold increase in respiration of the poisoned muscles on addition of the lactate ion. Addition of lactate to normal muscle causes an increase in respiration [Meyerhof, Lohmann and Meier, 1925], but the increase in oxygen uptake of poisoned muscle exceeds this by 50 p.c.

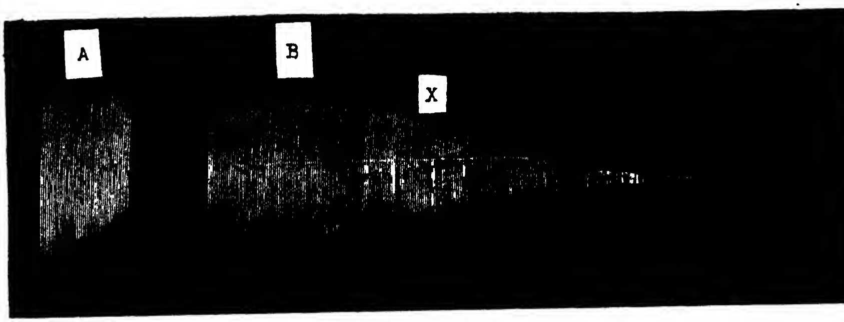

Fig. 1. Exp. 126. (Control for Exp. 128.) Sartorious in oxygenated Ringer solution. Ten single twitches per minute. *A*. Normal contractions. *B*. After soaking for 30 min. in 1/20,000 iodoacetate. At *X* rigor began after 113 twitches.

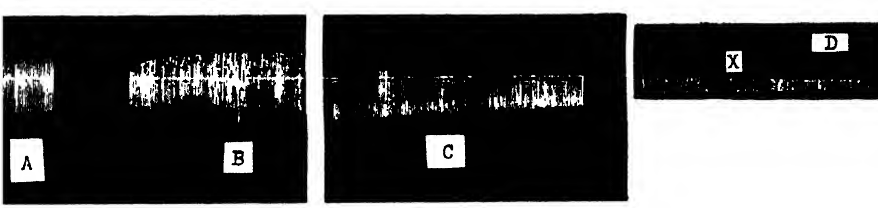

Fig. 2. Exp. 128. Sartorius in oxygenated lactate-Ringer solution. Ten single twitches per minute. *A*. Twitches in presence of lactate. *B*. First 100 twitches after 30 min. in 1/20,000 iodoacetate. *C*. Twitches from No. 500 to No. 650. *D*. Twitches from No. 1300 to No. 1440. Rigor began at *X*.

The question then arose as to the manner in which the muscle was using up its increased oxygen consumption. It seemed likely that the added lactate was being utilized as fuel for the provision of energy for an endothermic reaction, and it was possible that the most essential reaction in muscular metabolism—*i.e.* the resynthesis of creatine phosphoric acid, was being provided for in this way. It is well known that this resynthesis does not normally take place in muscle poisoned with iodoacetic acid [Lundsgaard, 1930]. If the combustion of added lactate in

oxygenated poisoned muscle did indeed result in the resynthesis of, perhaps, some part of the creatine phosphoric acid broken down, it would be expected that a poisoned muscle which received added lactate and oxygen would show powers of contraction superior in duration to those of a similar muscle supplied with oxygen alone.

This was, in fact, found to be the case. A solution of sodium *d*-lactate, prepared from cat muscle, was made up in phosphate-Ringer solution to a

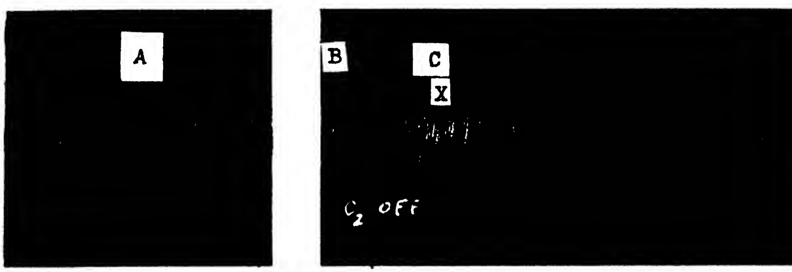

Fig. 3. Exp. 157. Sartorius in oxygenated lactate-Ringer solution. Six single twitches per minute. *A*. First 100 twitches after soaking in 1/20,000 iodoacetate. *B*. After 300 further twitches. *C*. Twitches in absence of oxygen after 15 min. anaerobic rest. At *X* rigor began, 25 twitches after resumption of stimulation.

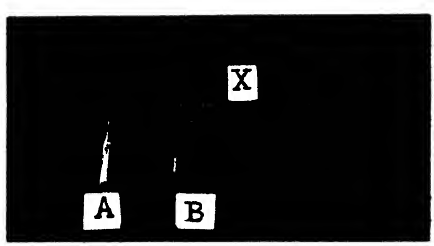

Fig. 4. Exp. 160. (Control for Exp. 157.) Sartorius in oxygenated Ringer solution. Six single twitches per minute. *A*. Normal contractions. *B*. After soaking for 30 min. in 1/20,000 iodoacetate. At *X* rigor began after 50 twitches.

concentration of about 0·04 p.c. lactic acid. A pair of sartorii of *Rana temporaria*, which had been poisoned by soaking for half an hour in sodium iodoacetate-Ringer solution (1/20,000 $CH_2ICOONa$), gave about 100 twitches in oxygen before rigor commenced (Fig. 1), but a sartorius which had been soaked for half an hour in lactate-Ringer before addition of sufficient iodoacetate to give the same concentration of poison would, after half an hour's soaking in the iodoacetate, give as much as 1400 twitches before signs of rigor began to appear (Fig. 2). In cases where

rigor developed at all in presence of lactate it was always much smaller in amount than in the poisoned muscle to which lactate had not previously been added.

The fact that this apparent protection of the muscle from the action of the poison is due to the oxidative removal of the added lactate, and not to interaction between the lactate and the iodoacetate as such, can be demonstrated by cutting off the oxygen supply (Fig. 3). The effect is not immediately visible owing to the amount of oxygen present in solution in the Ringer, but in a remarkably short time the muscle develops typical symptoms of iodoacetic acid poisoning, very soon ceases to twitch, and goes into rigor.

An attempt was made to obtain quantitative evidence of the disappearance of lactic acid from the solution during the activity of poisoned muscle, and for this purpose an apparatus was used which was suitable for the estimation of quantities of lactic acid of the order of 0·1 mg. [Mawson and Ritchie, 1932]. After the muscle had been at rest in oxygenated lactate-Ringer solution for half an hour a sample was withdrawn, suitably diluted, and the lactic acid estimated . Iodoacetate was added, the muscle left for a further half hour, and stimulation commenced. After some time another sample of the solution was removed and the lactate estimated again. Some typical results are given in Table VIII.

TABLE VIII.

Exp. No.	W (g.)	TL (kg.-cm.)	Weight of lactic acid (mg.) Before	After	Loss	$\frac{TL}{\text{Loss}}$
141	0·1540	182·3	1 630	1·415	0·215	848
145	0·1680	67·64	1·644	1·472	0 172	394
146	0·1612	44·36	1·650	1·528	0·122	364
148	0·1236	106·0	1·571	1·381	0·190	558

W = moist weight of muscle (g.).
L = length of muscle (cm.).
T = tension developed (kg.) during experiment.

The figures given are probably not sufficiently reliable to allow any conclusion to be drawn from the value arrived at for the relation $\left(\frac{TL}{\text{Loss}}\right)$ between the energy output of the muscle and the lactic acid consumed, but they are sufficient to demonstrate the removal of lactic acid by the poisoned muscle during a period of aerobic activity.

Several experiments were carried out in which creatine was added to the lactate-Ringer solution used in the other experiments, but it did not seem

to have any appreciable influence on the results obtained, and no disappearance of creatine from the solution could be detected on stimulation of the muscle.

It was shown by Lipmann and Meyerhof [1930] that the breakdown of creatinephosphoric acid was accelerated by raising the concentration of carbon dioxide in the muscle, so it would be anticipated that addition of CO_2 to the oxygen supply of a poisoned muscle in presence of lactate would tend to prevent the utilization of the lactate for the resynthesis of creatinephosphoric acid. It was found that the addition of up to 4·0 p.c. CO_2 (giving pH 6·8) to the oxygen supply had only a slightly deleterious effect, but any considerable increase of CO_2 tension above this value seriously hastened the onset of rigor, and resulted in a series of twitches similar to those given by a poisoned muscle working in the absence of added lactate.

A curious feature of these phenomena which is difficult to explain is that, once symptoms of poisoning have developed, the muscle cannot be saved. If, for instance, a muscle is first of all poisoned, and lactate added subsequently, although it will twitch for a somewhat longer period than if no lactate were added, the postponement of rigor and non-irritability is very limited, and this is also true, though to a less extent, if poison and lactate are added simultaneously. This may be due, at any rate in the former case, to the fact that even when the muscle is at rest its metabolic processes are slowly proceeding, and consequently the initially poisoned muscle is losing creatine phosphoric acid, without resynthesis, during its period of soaking. This point of view is strengthened by the fact that if a poisoned muscle, twitching vigorously in presence of lactate, is deprived for a few minutes of its oxygen supply, resumption of the passage of oxygen does not postpone for very long the development of marked symptoms of iodoacetate poisoning which would not, had the oxygen supply been continuous, have appeared until after several hundred further twitches.

It was also noticed that at temperatures below 10° C. poisoned muscles would continue to twitch for very much longer than they would, say, at 15° C. The initial part of the tracing of the muscle at low temperature was not greatly different from a trace taken at 15° C., but after about 100 twitches a very prolonged series of small, irregular twitches commenced which might continue to the number of three or four hundred. Rigor developed at about the hundredth twitch, as at 15° C., but it was very small in amount and did not increase nearly as rapidly as at the higher temperature.

It is not easy to relate the phenomena described to the metabolic processes of normal muscle, but it has been observed that part of the resynthesis of creatinephosphoric acid in normal muscle has to await the aerobic processes following the twitch [Hill, 1932]. It may be that this mechanism is, in poisoned aerobic muscle, called upon to bear the whole burden of resynthesis of creatinephosphoric acid. A poisoned muscle, even in the presence of lactate, although it will remain active much longer than if no lactate were present, becomes non-irritable long before a normal muscle would show serious signs of fatigue, and this may well be due to the fact that the synthetic system kept in action by combustion of lactate is insufficient to keep up the store of creatinephosphoric acid to its normal level, with the result that constant wastage occurs. It must not be forgotten, too, that poisoned muscle is continuously losing glycogen, and abnormal amounts of hexose phosphoric esters are accumulating.

It is, however, clear that the reason for the rapid deterioration of poisoned muscle, even in oxygen, is the actual absence of lactic acid, and that the non-production of lactic acid is not merely a symptom of the conditions. The results also tend to strengthen the point of view that lactic acid is an intermediate step in the oxidation of glycogen to carbon dioxide and water, rather than the theory that lactic acid lies off the main path of carbohydrate oxidation.

The point of action of the iodoacetate is evidently not at the end of the chain of events, and as we know that the beginning of the chain is also unaffected by the poison the broken link may be sought in a more limited field. The suggestion of Meyerhof [1926] that the appearance of hexose phosphoric esters is due to abnormal stabilization of a labile hexose monophosphoric ester is very attractive, but would seem to be difficult, from its nature, of verification.

I wish to acknowledge a private communication from Dr E. Lundsgaard, who informs me that he has recently carried out experiments on the addition of lactate to oxygenated iodoacetate muscle with results similar to those reported in this paper.

SUMMARY.

Part I.

No anaerobic disappearance of lactic acid takes place in a muscle poisoned with iodoacetic acid.

Part II.

1. A muscle poisoned with iodoacetate in the presence of oxygen and lactate will continue to contract long after a similar muscle, without added lactate, has become non-irritable and has gone into rigor.
2. Lactate disappears during such a prolonged series of twitches, and it is suggested that it is used, at least in part, as fuel to provide for the resynthesis of creatinephosphoric acid.
3. Addition of more than 4 p.c. of CO_2 to the oxygen supply tends to prevent the prolongation of activity due to added lactate.
4. The disappearance of added creatine during the activity of aerobic iodoacetate muscle could not be demonstrated.

In conclusion I wish to acknowledge a grant from the Medical Research Council and to express my gratitude to Mr A. D. Ritchie, M.A., for his invaluable advice and encouragement, and to Prof. H. S. Raper, F.R.S., for his assistance in the revision of this paper.

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REVERSAL OF THE GASTRIC VAGUS.

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GARRY [1930 *a*, *b*] and Shafer, Underwood and Gaynor [1930] found that amytal (iso-amyl ethyl barbituric acid) had the peculiar property of removing the inhibitory effect of the vagus on the heart. The effect was demonstrated in animals anæsthetized with amytal and in decapitate preparations which had received subanæsthetic doses of the drug by intraperitoneal injection or by intravenous infusion.

It appeared to us that considerable interest would attach to the investigation of the action of the drug on the response of the stomach to vagus stimulation. There exists a considerable body of very contradictory literature with reference to the effect on the stomach of the vagus. This has recently been reviewed by McSwiney [1931]. In general it appears that both excitation and inhibition may result from vagus stimulation, a factor in determining the direction of the response being the state of activity of the stomach at the time of stimulation. The active contracted stomach is stated to respond by relaxation, and the relaxed organ by contraction [Carlson, Boyd and Pearcy, 1922; McCrea, McSwiney and Stopford, 1925; McCrea and McSwiney, 1926]. A number of observers have found that the frequency and strength of the stimulation may play a part in determining the effect. Thus, Veach [1925] is of the opinion that the effect of the vagus on the lower œsophagus and stomach is of the nature of Wedensky inhibition. Veach, Schwartz and Weinstein [1930] have suggested the term "inhibition by fatigue" as being more applicable to the effects of the vagus on the stomach. McSwiney and Wadge [1928] have brought forward evidence to disprove the existence of Wedensky effects.

We hoped by the use of a drug such as amytal, which appeared to have a specific action on the vagal inhibitory mechanism, to elucidate some of the problems presented by these conflicting views.

Methods.

Cats were employed throughout the investigation, since they afforded a preparation which is reliable and which gives consistent results. In many experiments the spinal cat was used: it was prepared under ether by transection of the cord at the atlanto-axial joint and destruction of the brain with sinus forceps. Artificial ventilation on oxygen was given

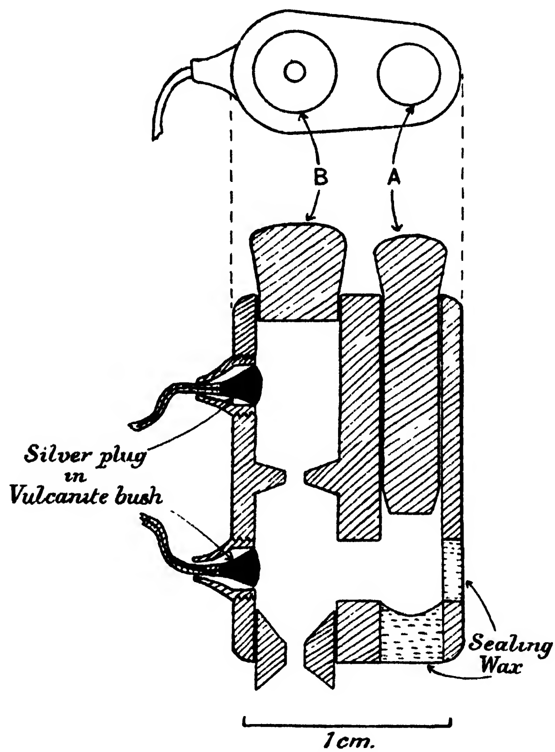

Fig. 1. Diagram of vulcanite fluid electrode.

by a Starling pump; the ventilation was kept as low as possible compatible with adequate arterialization of the blood, and a sufficient dead space was provided.

After preparation of the animal, the pylorus was ligated through a small abdominal incision, the abdomen was closed, and the stomach was washed out through a tube passed through a cervical œsophagotomy. In those experiments in which it was wished to secure activity of the

stomach, 60 c.c. of meat extract in warm water were administered immediately after washing out. A water float manometer connected to the stomach tube was used for recording the gastric movements, the whole system being filled with warm 0·9 p.c. saline. The peripheral end of the cut vagus nerve was stimulated both in the neck and below the heart in the thorax, and, in certain experiments, the thoracic sympathetic chain was also stimulated. To expose the infracardiac vagus, the ribs were exposed through a longitudinal incision through the skin and latissimus dorsi, an intercostal space was opened and the ribs held apart. One of the larger branches of the vagus was identified on the œsophagus, tied and dissected downwards for some 2 cm. The œsophageal vagus in the cat consists usually of a main right and left branch with one or more finer twigs lying posteriorly. When necessary, the sympathetic chain was dissected out through the same incision.

All nerve stimulation was carried out by means of fluid electrodes filled with the warmed defibrinated blood of the animal under experiment. The electrodes were constructed of vulcanite, electrical contact being made by silver plugs (Fig. 1). The nerve is drawn through on a fine ligature and the lower hole filled with vaseline or a mixture of hard paraffin and vaseline. The lower chamber is then filled with blood by means of the side tube *A*, which is then plugged. The upper chamber is filled through *B*, and the thread holding the nerve is secured by the plug. After application to the nerve, the electrodes are allowed to rest in the thorax, and the thoracic wall is closed. In these conditions the nerve retains its excitability practically unaltered for as long as 6 hours, spread of current is effectively eliminated, and the nerves may be stimulated without any interference with the animal under experiment. Stimuli of frequencies between 2 and 60 break shocks per sec. were applied by means of a calibrated induction coil and the contact breaker described by Brown and Lees [1931].

Results.

In all experiments in which amytal was administered to the spinal animal, the normal reaction to vagus stimulation was determined prior to the administration of the drug. We consider that the discrepancies in the literature and the rarity with which animals not under the influence of anæsthetics have been employed for the investigation of the effect of vagus stimulation on the stomach justify a separate consideration of the results obtained.

Normal response to the vagus.

The account given below applies to the effects of stimulating both the cervical and the infracardiac vagus unless statement is made to the contrary. In general, the results may be divided into three classes.

(*a*) When the stomach shows definite tone and activity. As a criterion of the tonus of the stomach we have used the ability of the organ to maintain a head of pressure in the manometer [McCrea, McSwiney and Stopford, 1925]. When the stomach is atonic, the introduction of as much as 200 c.c. of fluid does not cause the entogastric pressure to rise above about 5 cm. When, on the contrary, the gastric tonus is high, the stomach will maintain a pressure of as much as 15 cm. with the introduction of only 60 c.c. of fluid. The term activity we have used in reference to the presence of rhythmic movements. Adequate rhythmic movements are usually, but not always, associated with good tone.

When the stomach shows good tone and activity, the response to vagal stimulation is predominantly one of inhibition (Fig. 2 A). Frequently the inhibitory effect is preceded by a sharp contraction of short duration. In some instances, with prolonged stimulation, the inhibition tends to escape and the record regains its previous level. The inhibition is sometimes followed by some augmentation of rhythmic movements and a rise of the record above its previous base line. In experiments of this class, inhibition results from all strengths and frequencies of stimulation, a frequency as low as 2 per sec. causing a profound and lasting inhibition.

(*b*) When the stomach is definitely atonic. The response to vagus stimulation is predominantly one of contraction and augmentation or initiation of rhythmic movements. The contraction is frequently preceded by a relaxation of very short duration.

(*c*) When the condition of the stomach is indefinite, motor responses are common at the outset of the experiment. This response frequently becomes more and more of the nature of an inhibition as the stimulation is repeated and the tone and activity rise. In these experiments, variations in the response due to alterations in frequency, duration and strength of the stimulating current are most commonly observed. In the majority of our experiments, low-frequency stimulation favoured inhibition and high-frequency favoured contraction. In one experiment alone, results which might agree with the "Wedensky" hypothesis of Veach [1925] were observed. The effects of duration of stimulation are of interest. A short duration stimulus of high frequency produced

inhibition as a post-stimulation phenomenon. Prolongation of such a stimulus carried the response through the inhibition to a marked contraction. It is, indeed, frequently possible to obtain any response desired by the adjustment of the duration and type of stimulation. This is rendered especially easy when the final response, be it contraction or relaxation, is preceded by an initial response of short duration of the opposite sign. For instance, relaxation is, as previously pointed out,

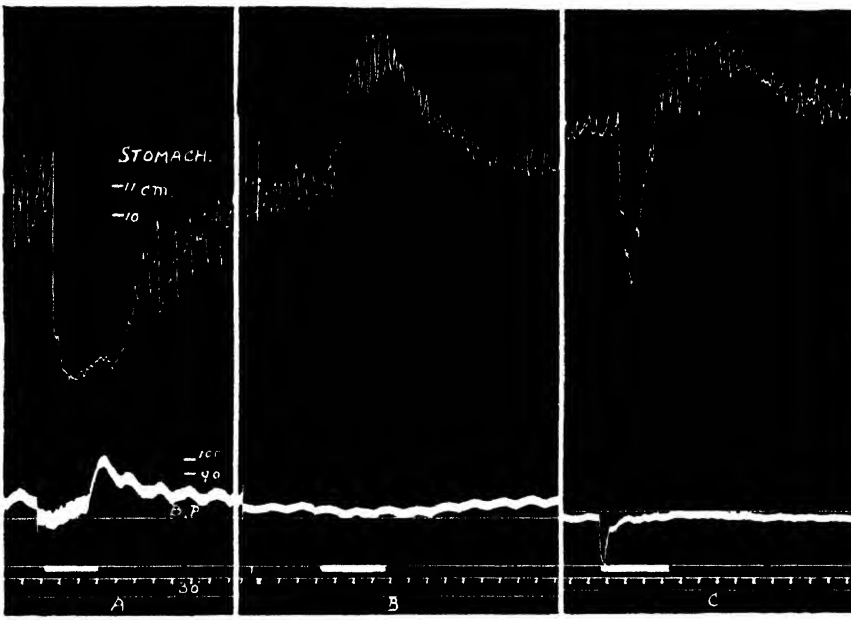

Fig. 2. Spinal cat. Stimulation of left vagus in the neck: A. Before amytal, 50 per sec., coil 0. B. Immediately after administration of 40 mg. of amytal per kg., 50 per sec., coil 0. C. Three hours after amytal, 50 per sec., coil 0.

frequently preceded by a sharp contraction; suitable stimulation at a sufficiently low frequency will then produce a series of such preliminary contractions, giving the appearance of a true motor response. Similarly, as shown above, a short stimulation may evoke only the short relaxation which frequently precedes an otherwise pure motor reaction.

In general, no significant differences were observed between the effect of stimulating the vagus in the neck and below the heart, and, in fact, the records obtained from each site of stimulation are frequently superimposable. In one experiment alone, neck stimulation produced contraction and thoracic relaxation.

To summarize, then, it appears that the predominant factor determining the direction of the reaction of the stomach to vagus stimulation is the condition of activity and tonus of the organ. Where this is indeterminate, the response may be controlled by the frequency and duration of the stimulating current. In many experiments, low frequency stimulation is more favourable to relaxation than high frequency. It is of interest to note in this connection that Dickinson and McSwiney [1932] find that in the isolated innervated preparation of the tortoise stomach, low-frequency stimulation is always more effective in the production of an inhibitory response to vagus stimulation.

Effects of amytal.

(1) *In the spinal animal.*

(*a*) *Vagus response in the neck.* The initial experiments were performed in order to find whether amytal had any effect on the motor response of the stomach to vagus stimulation. Accordingly, spinal cats were prepared, and the administration of meat extract after ligation of the pylorus was omitted. In these circumstances, the usual response to vagus stimulation is one of contraction. The administration of amytal did not interfere in any way with the augmentor effect of vagus stimulation.

Preparations were then made for the study of the inhibitory vagus effect. A constant inhibitory response to vagus stimulation was first established. Amytal was then either injected intravenously in small volume or given in dilute solution by slow intravenous infusion. The immediate effect of the drug is to cause a considerable fall of blood-pressure, which later recovers, and frequently a rise in tone of the stomach. This rise of stomach tone is most frequently seen after small doses. Doses of an anæsthetic value may cause some depression of gastric tone and activity.

After administration of the drug, vagus stimulation of all strengths and frequencies causes contraction of the stomach (Fig. 2), the previous inhibition being completely reversed. This contraction may be preceded by a small preliminary inhibition. Fig. 2 also shows the partial disappearance of the action of the drug after the passage of 2 hours. It is interesting to note that the reappearance of the gastric inhibitory effect is synchronous with the reappearance of the cardiac inhibition. It must be pointed out, however, that the effects of the drug on the cardiac vagus and on the gastric vagus do not necessarily run parallel, since reversal of the gastric vagus may be produced by doses of the

drug insufficient to abolish the inhibitory effect of the vagus on the heart.

(*b*) *Vagus response in the thorax.* It appeared possible that the reversal of the gastric vagus effect might be connected in some way with the abolition of the inhibition of the heart. We accordingly stimulated

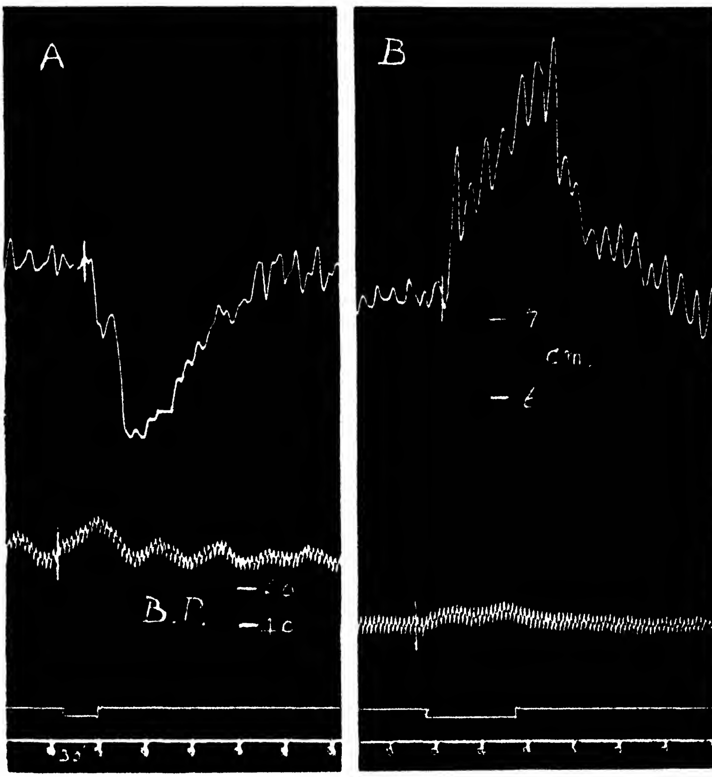

Fig. 3. Spinal cat. Stimulation of right infracardiac vagus: A. Before amytal, 60 per sec., coil 10. B. After 30 mg. of amytal per kg., 60 per sec., coil 10.

the vagus below the heart before and after the administration of amytal. Fig. 3 shows the effect on the vagus response of such an injection of amytal: there is, as in the neck, complete reversal of the inhibitory effect. The reversal of the infracardiac vagus is, however, not always as clear cut as the reversal from stimulation of the cervical vagus. Preliminary inhibitions are more prominent and persist after large doses of the drug.

(2) *In the animal anæsthetized with amytal.*

Stimulation of the cervical vagus in the animal anæsthetized with amytal caused in all cases contraction of the stomach. No inhibitory effects were observed. When the vagus is stimulated below the heart, a very interesting result is observed (Fig. 4). In the majority of experiments, the first stimulation in the thorax is definitely inhibitory. On repeating the stimulation when the stomach record has reached its previous level, the response is one of diminished relaxation or even

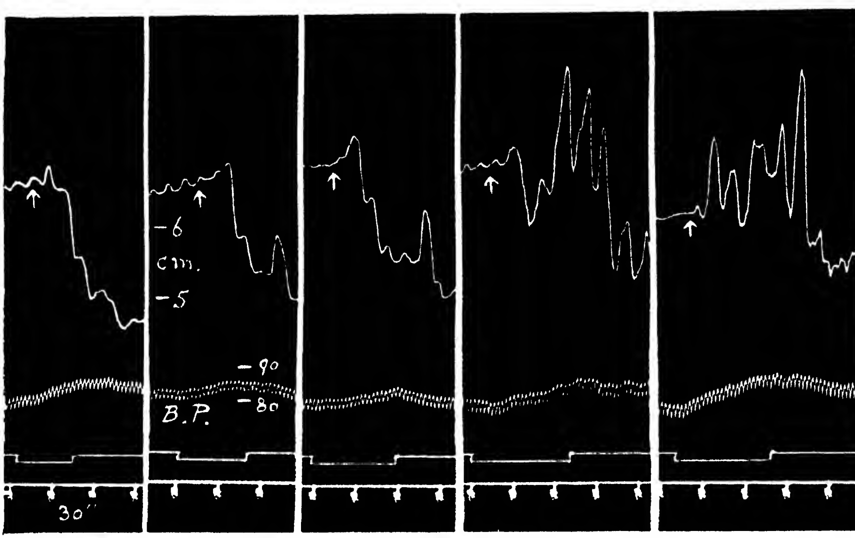

Fig. 4. Cat anæsthetized with amytal. Consecutive stimulations of left infracardiac vagus, 10 per sec., coil 10; showing development of reversal.

contraction. With succeeding stimulations, the inhibitory effect becomes successively smaller until pure contraction alone is the final result of stimulation. Once the motor effect has been elicited, it persists throughout succeeding stimulations, if they are given in close sequence. If, however, no stimulation be applied for a period of at least 20 min., the first stimulation after this interval causes inhibition, contraction again occurring on repeating the stimulation.

Amytal and the sympathetic.

The action of amytal appears to be restricted to the inhibitory vagal mechanism of the stomach. In animals anæsthetized with the drug and in spinal animals which have received amytal, stimulation of the thoracic

sympathetic chain causes inhibition with high frequency stimuli, while the vagus both in the neck and in the thorax causes contraction (Fig. 5). It must, however, be pointed out that amytal, like all anæsthetics, may, in large doses, produce sufficient depression of the stomach to reverse

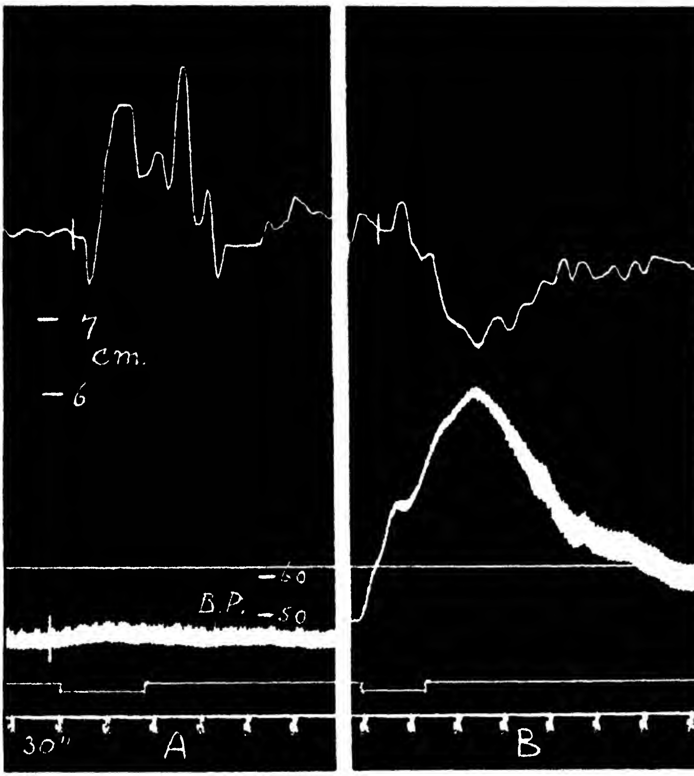

Fig. 5. Spinal cat after administration of 62 mg. of amytal per kg.: A. Stimulation of left cervical vagus, 60 per sec., coil 7. *B.* Stimulation of right thoracic sympathetic chain, 60 per sec., coil 10. Previous to administration of the drug, both nerves produced relaxation.

the action of the sympathetic. If amytal in dilute solution be allowed to infuse slowly into a vein, the complete sequence of the reversal may be observed. At the beginning of the experiment, stimulation of both nerves causes relaxation of the stomach. As the concentration of amytal rises, the response to vagal stimulation becomes contraction, the sympathetic remaining inhibitory. Further increase of the drug concentration causes reversal of the sympathetic.

Discussion.

The response of the stomach of the spinal animal to vagus stimulation has been investigated with a view to the establishment of the normal reaction. It appears clear that, as far as our present knowledge goes, the main factor determining the direction of the response of the stomach to vagus stimulation is the condition of activity of the peripheral mechanism. The frequency of stimuli applied to the nerve may play a part in determining the response, but only when the peripheral conditions are, as it were, in the balance. In these circumstances, frequencies between 2 and 20 per sec. are most effective in causing inhibition. We have found no evidence in favour of the existence of Wedensky effects in our preparations.

We wish to draw attention to the very significant differences which exist between the response of the stomach to vagus stimulation and the effects of sympathetic stimulation. As Brown, McSwiney and Wadge [1930] have shown, the response of the stomach of the spinal animal to sympathetic stimulation bears little relation to the activity of the peripheral mechanism, the main factor being the frequency of stimuli incident upon the nerve. This difference is very well shown in the behaviour of the isolated innervated stomach preparation. McSwiney and Robson [1931 *a*, *b*] and Brown and McSwiney [1932] were able to obtain inhibition and contraction of the stomach strips at will by altering the frequency of stimuli applied to the peri-arterial (sympathetic) nerves. In the case of the vagal innervated strip, contraction was readily obtained, but inhibition occurred only after the addition of atropine to the preparation [McSwiney and Robson, 1929].

Further very striking differences between vagus and sympathetic effects are observed in the form of the reactions in the intact animal. The inhibitions and contractions evoked by sympathetic stimulation are almost invariably simple in form. Those produced by vagus stimulation are almost as frequently complicated by preliminary reactions opposite in sign to the final response. A striking feature of these precursory reactions is their phasic character, the total duration being seldom more than 30 sec. These preliminary effects add considerably to the difficulty of interpretation of records, since stimuli of short duration may be sufficient to elicit only the preliminary effect.

It is clear, then, that the vagus and the sympathetic effects on the stomach present many gross differences. In general it appears that the vagus system is very much more sensitive to external influences than

the sympathetic, and that the vagal inhibitory mechanism is largely disabled by the environmental change incident in the preparation of the isolated innervated strip. It is tempting to attribute these differences to the presence of the peripheral neurone in the vagus system.

The effects of amytal on the response of the stomach to vagus stimulation present several points of interest. The gastric peripheral mechanism is peculiarly susceptible to depressant drugs, and persistent motor effects are not uncommon in anæsthetized animals. The action of amytal, however, appears to be much more specific than due simply to general depression of gastric function. In spinal animals which have received small doses of the drug, there may be an actual increase in tonus and activity, and, moreover, in animals anæsthetized with the drug, the initial reaction to vagus stimulation in the thorax may be inhibitory.

It has been suggested that the inhibitory effects on the stomach of stimulation of the cervical vagus might be associated with the effect of the vagus on the heart, either mechanically on account of the fall of general blood-pressure, or by transmission by the blood stream of specific substances liberated in the heart [Brinkmann and Van der Velde, 1925]. The normal response of the stomach is apparently identical whether the vagus be stimulated in the cervical region or below the heart. There exist, however, certain differences when the effects of amytal are recorded. It appears that it is more difficult to reverse the vagus with amytal when stimulation is carried out below the heart. This phenomenon, however, does certainly not suggest that the cardiac effects do in any way contribute directly to the gastric inhibition.

The limitation of the action of amytal to the vagal system is of interest. Brown and McSwiney [1932] have recently shown that the inhibitory effects on the stomach of sympathetic stimulation may be reversed by the administration of luminal, and that the reversal is confined largely to the sympathetic system, since when the action of the sympathetic is purely motor, inhibition can still be obtained by vagus stimulation. In the case of amytal, the reverse holds good. When the vagus is causing contraction only, sympathetic stimulation still produces relaxation. It must be pointed out, however, that, with both drugs, pushing of the dose too far can produce abolition of the inhibitory effects of both the vagus and sympathetic.

The present work shows that there exist very marked differences both in the response of the stomach to vagus and sympathetic stimulation and in the relative susceptibility of the two systems to certain anæsthetics. Kiss [1931] has recently suggested that the vagus conveys

to the stomach and intestines fibres which are entirely sympathetic in origin, and that the actions of the so-called parasympathetic system are merely a "negative phase" of true sympathetic activity. While we are not in a position to criticize Kiss's morphological findings as to the central origin of the anatomical vagus, this work shows that the peripheral effects, at least, of the two systems are very different and suggest a distinct mechanism for their actions. The same criticism is applicable to the work of Kuré [1930, 1931], in that he suggests that the motor effects of splanchnic stimulation are due to admixed fibres of parasympathetic origin.

Brown and McSwiney [1932] have suggested that the motor and inhibitor responses of the stomach to the sympathetic and their reversal by luminal might be explained on the assumption of the liberation peripherally of a specific substance, the direction of response depending on the rate of liberation.

We have not sufficient evidence at our disposal to be able satisfactorily to explain the vagus phenomena on the same basis. It is, however, significant that we have found the optimum frequency of stimuli to produce inhibition by the vagus to be between 2 and 20 per sec., in view of the findings of McSwiney and Robson [1929] that maximum summation of stimuli takes place in the vagal peripheral neurone when the shocks applied to the nerve fall at an interval of 0·1 sec. This suggests that inhibition in the intact animal is associated with the maximum peripheral effect.

SUMMARY.

The normal reactions of the stomach of the spinal cat to stimulation of the cervical and infracardiac vagus are described. Stimulation may result in either contraction or relaxation. The predominant factor determining the direction of response is the condition of activity of the peripheral neuro-muscular mechanism. When this is indeterminate, the direction of response may be controlled by alteration in the frequency and duration of the stimulation; stimuli falling between 2 and 20 per sec. are most effective in producing relaxation. No significant differences were observed between the effects of vagus stimulation in the neck and below the heart.

Administration of amytal (iso-amyl ethyl barbituric acid) in subanæsthetic doses reverses the inhibitory action of the vagus, leaving unaffected the response to sympathetic stimulation. The differences

between the effects on the stomach of vagus and of sympathetic stimulation are discussed.

We wish to express our thanks to Prof. B. A. McSwiney for his advice and criticism throughout this investigation.

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deeper part of the incision where there is great danger of perforating blood vessels of the vascular system serving the ventral part of the head.

Ligatures are now placed around the origin of the ventral aorta and around the posterior part of the bulbus but not tied, and the bulbus is partly severed by a transverse scissors' cut midway between the two ligatures. After preliminary washing of the wound with Ringer's solution, the cannula, attached to the perfusion system and slowly dripping

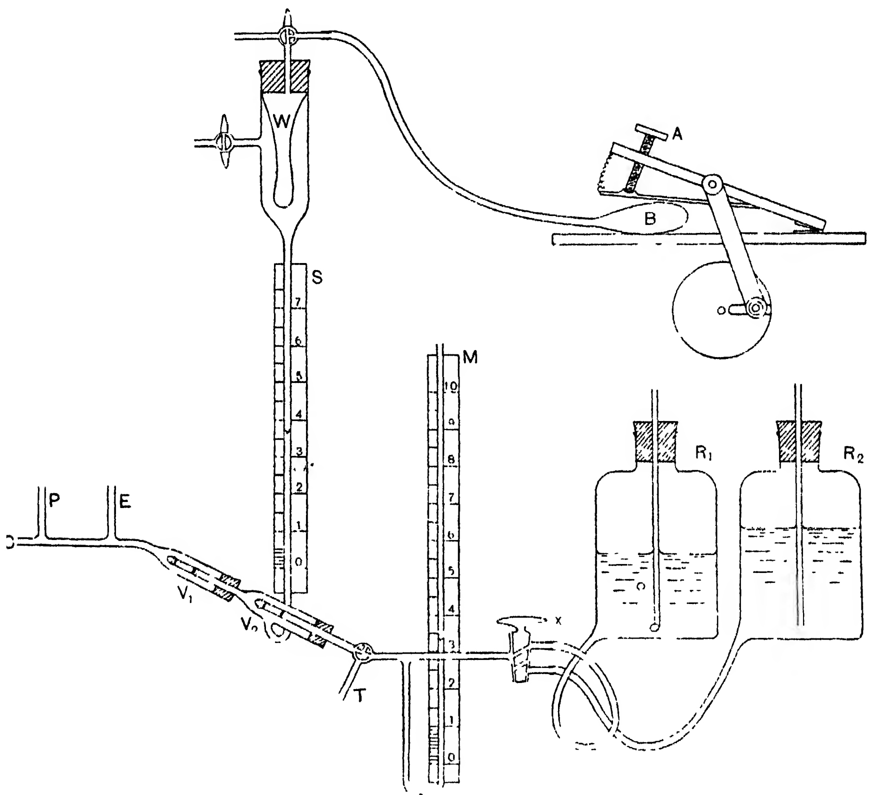

Fig. 1. Apparatus arrangement for perfusion of gills with pulsating pressure. Designed for rates of flow between 10 and 2000 c.c. per hour. See text for explanation.

perfusion fluid, is inserted into the ventral aorta through the opening in the bulbus and perfusion is begun. When satisfactory perfusion is demonstrated (by the appearance of a good flow from the dorsal aorta), the ligatures are tied and the incision in the body wall is closed around the cannula with sutures and wound clips. Speed in establishing the branchial circulation is, of course, essential.

The remainder of the operation, cannulation of the dorsal aorta, etc., may be completed as in the heart-gill preparation. For many purposes the closed external circulation may be dispensed with as it has been shown [Keys, 1931 *b*] that the total chloride loss from the internal system is equivalent to the total gain of the external system.

Chloride concentrations were determined by Keys' [1931 *c*] method, while vapour pressures were determined by Hill's [1930] method in the manner described by Margaria [1930]. The perfusion fluid was the same as that used in the previous perfusion experiments with eels except that urea was omitted[1].

Vapour-pressure determinations on the perfusion medium of the concentration adopted for the present work indicated it to be the osmotic equivalent of a 1·196 p.c. solution of NaCl; in other words its concentration amounted to 0·3179 osmoles per litre.

Experimental results.

The principal experimental results are presented in Table I, in which the chloride and vapour-pressure changes in the perfusion fluid resulting from repeated circulation through the heart and gills (Group A) and through the gills alone (Group B) are given in comparable terms. It should be noted that all the eels had been acclimatized to sea water for some days before experiment and the gills were bathed in flowing sea water (freezing point − 2·0° C.) throughout the experiments.

Table I.

Exp No	Live wt of eel (g)	Hours after start of operation: Begin exp period	End	Mean vol internal medium (c.c.)	Chloride concentrations (mg./100 c.c.): Begin	End	Vapour-pressure change as equivalent to p.c. NaCl	Concentration changes calc. as net mg. chloride transferred per hour/kg of eel from: Cl determinations	Vapour-pressure determinations
1 A*	280	1·22	2·66	43·4	692·5	—	+0·026	—	+17
2 A	411	1·05	2·47	63·5	692·3	682·2	+0·007	−10·0	+ 4
3 A	454	1·00	2·87	83·6	692·3	686·7	+0·0157	− 5·5	+ 9
1 B	450	1·20	3·07	63·2	692·3	673·6	−0·0127	−13·7	− 7
2 B	322	0·75	2·42	67·8	692·3	684·4	−0·0102	− 9·9	− 8
3 B	438	0·80	1·73	69·2	692·3	685·4	−0·0115	−11·7	−12
4 B	345	0·72	2·93	69·2	692·3	680·0	−0·0124	−11·2	− 7
5 B	350	0·90	2·10	69·2	692·3	691·1	−0·005	− 1·8	− 4
6 B	234	1·42	4·12	69·2	692·3	687·1	−0·023	− 5·7	−15
7 B	281	0·92	2·38	69·2	693·0	691·1	+0·0019	− 3·2	+ 1

* Gill surface slightly injured in this experiment.

A representative protocol from each group will serve to illustrate the manner in which the foregoing results were obtained.

[1] 20 c.c. $CaCl_2$ 0·149 M., 30 c.c. KCl 0·196 M., 0·40 g. $NaHCO_3$, 0·90 g. glucose, made up to 1 litre with 0·1960 M. NaCl.

Protocol 3 A. *Heart-gill repeat circulation.*

Eel from sea water (5 days), weight 454 g.
Operation begun 11:08 a.m., internal circulation established 11:20 a.m., operation complete 11:58 a.m.
Internal medium standard perfusion medium, Δ about 0·71° C.
External medium pure sea water, Δ about 2·0° C.
Temperature of gills 11° C.
Control sample of circulating perfusion medium taken at 12:09 p.m.
Experiment ended at 2:00 p.m. and sample of perfusate taken for analysis.
Mean volume of perfusion medium in circulating system 83·6 c.c.
Weight of head at start of experiment 157·5 g.
Chloride concentrations:

Control

692·32, 692·26, 692·30 } Mean 692·3 mg. per 100 c.c.

Experimental

686·76, 686·62, 686·78, 686·64 } Mean 686·7 mg. per 100 c.c.

Vapour-pressure determinations:

Concentration change expressed in terms of an osmotically equivalent NaCl solution.

Experimental solution = control + 0·0135, + 0·0159, + 0·0165, mean + 0·0157 p.c. NaCl.

Calculations:

$$\text{I.}\quad \frac{(-5{\cdot}6)\,(0{\cdot}836)}{(2{\cdot}87-1{\cdot}00)\,(0{\cdot}454)}=-5{\cdot}5,$$

mg. Cl lost per hour per kg. of eel indicated by chloride determinations.

$$\text{II.}\quad \frac{(+0{\cdot}0157)\,(35{\cdot}5)\,(0{\cdot}836)}{(2{\cdot}87-1{\cdot}00)\,(58{\cdot}5)\,(0{\cdot}454)}=+9,$$

or total osmotic concentration gain expressed as mg. Cl per hour per kg.

Protocol 1 B. *Ventral aorta-gill preparation, repeat circulation.*

Eel from sea water (3 days), weight 450 g.
Operation begun 11:36 a.m., internal circulation established 11:47 a.m., operation complete 12:15 p.m.
Internal medium standard perfusion medium, Δ about 0·71° C.
External medium pure sea water, Δ about 2·0° C.
Temperature of gills 15° C.
Control sample taken at 12:55 p.m. (1·20 hours after start of operation).
Experiment ended at 2:40 p.m. (3·07 hours after start of operation) and perfusate sample taken.
Mean perfusion volume 63·2 c.c.
Chloride concentrations:

Control

692·31, 692·43, 692·23, 692·36 } Mean 692·33 mg. Cl per 100 c.c.

Experimental

673·51, 673·67, 673·62, 673·72 } Mean 673·63 mg. Cl per 100 c.c.

Vapour pressures:

Experiment = control − 0·0165, − 0·0123, − 0·0092, mean − 0·0127 p.c. NaCl.

Calculations:

$$\text{I.}\quad \frac{(-18{\cdot}7)\,(0{\cdot}632)}{(3{\cdot}07-1{\cdot}20)\,(0{\cdot}0450)} = -13{\cdot}7,$$

mg. Cl lost per hour per kg. as indicated by chloride determinations.

$$\text{II.}\quad \frac{(-0{\cdot}0127)\,(935{\cdot}5)\,(0{\cdot}632)}{(58{\cdot}5)\,(3{\cdot}07-1{\cdot}20)\,(0{\cdot}450)} = -7,$$

or total osmotic concentration loss expressed as mg. Cl loss per hour per kg., calculated from vapour-pressure determinations.

The figures for the concentration changes as indicated by the vapour-pressure determinations are subject to a relatively large error. Even with the most careful procedure the error in the vapour-pressure determinations is rarely less than $\pm 0{\cdot}002$ p.c. NaCl in the concentration differences with which we are dealing. The magnification of the concentration changes produced by the repeat circulation is limited by the loss of perfusion medium by leakage. In spite of every effort to reduce this leakage to a minimum, we were unable to reduce it below about 3 p.c. of the total circulation in the heart-gill preparation and about 5 p.c. in the ventral aorta-gill preparation. While there was no difficulty in preventing leakage around the points of cannulation, there remained two sources of loss. Leakage from the cut edges of the tissues is only partly prevented by such expedients as reducing the incised tissue to a minimum and closure of the incisions with sutures and clamps; the other, and more important source of loss of perfusion fluid, is through leakage into the tissues of the head, producing œdema. We have made a number of measurements of the amount of œdema.

There are two forces tending to cause œdema in the perfusion experiments. Communication with the tissues of the head by way of the arterioles is not entirely blocked, although return of fluid from these tissues to the perfusion system is impossible. The head tissues will thus be subjected to an effective hydrostatic pressure practically equal to the full arterial pressure, and the pressure available tending to cause filtration through the capillary walls will be correspondingly greater than in the normal animal where there is a free venous outflow [see Krogh, Landis and Turner, 1932]. The reduction in pressure afforded by the relatively insignificant venous escape from the cut edges of the tissues in the incision can only be slight, so that the venous pressure will tend to approximate to the arterial pressure.

The second factor tending to cause œdema in the tissues of the head is the colloid osmotic pressure of the tissues. In the normal vertebrate animal an equilibrium, or at least a steady state, exists in which the tissue volume and water content remain constant when the blood has a

colloid osmotic pressure of something like 20 to 30 mm. Hg [see Krogh, 1929, p. 293 *et seq.*], and we take it that the tissue colloid osmotic pressure is not dissimilar. The perfusion fluid used in the present experiments contained no colloid, this being omitted because the high concentrations necessary to produce osmotic pressures of the order required interfere seriously with the chloride determinations.

The measurements of œdema were made by weighing the severed head before and after perfusion as a repeat circulation ventral aorta-gill preparation (see Table II).

TABLE II. Œdema in the perfused head of the eel.

Initial weight of head (g.)	Final weight (g.)	Duration of exp. (hours)	Œdema fluid (c.c.)	Œdema fluid as p.c. of initial weight
157·5	174·0	1·87	16·5	10·5
116·0	137·5	1·67	21·5	18·6
135·0	165·0	0·93	30·0	22·2
88·0	125·0	2·11	37·0	42·0
101·0	123·5	1·20	22·5	22·3
79·5	89·0	2·70	9·5	11·9
93·0	108·0	1·46	15·0	16·1

Performance of the heart in the repeat circulation heart-gill preparation.

Before discussing the bearing of the experimental observations on the problem of the secretory activity of the gills, some mention should be made of the performance of the heart in the repeat circulation heart-gill preparation. In the ordinary heart-gill preparation where fresh perfusion medium is supplied to the heart continually the heart beat, with the exception of a short period immediately following the operation, is extremely regular in rate. The cardiac output is also quite steady. Observations made by one of us (A. K.) in Copenhagen showed that it is quite usual for the heart rate to vary less than 5 p.c. for some hours, up to 10 hours in some cases, although the heart became obviously distended relatively early in the experiment. That the output is not so constant is not surprising in view of the fact that it was found that the output of the eel's heart is extremely sensitive to slight changes in the "venous" pressure at which the internal medium is supplied to it. For example, it was found that changes of less than 0·5 mm. Hg in venous pressure might double or halve the output, and a change of as little as 0·1 mm. Hg frequently had a measurable influence[1]. With these facts in mind the

[1] Note, however, that the optimal venous pressure for the heart-gill preparation is very low, being of the order of 3 mm. Hg [see Keys, 1931 *a*, p. 361], so that a change of 0·1 mm. Hg amounts to over 3 p.c. of the total venous pressure.

performance of the heart under the conditions of the repeat circulation may be discussed.

The repeat circulation experiments were always started as a single circulation, and repeat circulation not begun until the heart beat had become thoroughly strong and regular and the perfusate practically free of blood (usually about half an hour for the latter condition). Following the change over to repeat circulation signs of marked failure of the heart were usually apparent within the hour; in a number of cases the heart stopped completely in less than an hour and could not be revived by mechanical stimulation or warming. This could not have been due to lack of oxygen, as the perfusion medium was continuously aerated with air which was previously bubbled through a 1·5 metre column of perfusion medium. Excess acidity due to the release of CO_2 into the medium by metabolism is out of the question, as the medium maintains a *p*H of about 7·6 when in contact with an atmosphere containing 5 p.c. CO_2 and very slowly tends to become alkaline when the partial pressure of carbon dioxide is less than this amount.

The possible causes of this effect of the perfusate when reintroduced into the heart then seem to be reduced to the following:

(1) Abnormal alkalinity due to the spontaneous shift in the medium in the alkaline direction associated with the slow dehydration of H_2CO_3 [see Ahlgren, 1929; Keys, 1931 *a*, p. 359; Brinkman, Margaria and Roughton, 1931]. But perfusion medium which had been well shaken with air and allowed to stand for some hours before use did not affect the heart when passing through it for the first time.

(2) Branchial embolism and resultant high back pressure on the heart due to coagulation of traces of blood washed out of the gills. But addition of heparine to the medium did not prevent the heart failure ensuing on several cycles of repeat circulation. Moreover, it was found that the heart could be almost completely revived by the introduction of fresh medium and would then maintain a high rate of perfusion.

(3) Exhaustion of the glucose content of the medium. Sugar determinations (method of Hagedorn and Jensen) showed this not to be the case.

(4) Change in the concentration of the inorganic salts in the medium. Calcium determinations (by the kindness of Miss E. Watchorn), as well as the chloride determinations and the vapour-pressure measurements, eliminated the probability of any appreciable effects from this direction.

(5) Washing out of some essential substance from the heart muscle.

But this would be just as effectively accomplished by the single perfusion arrangement.

(6) Poisoning of the heart by some toxic substance or substances produced by itself or by the tissues of the gills. By the process of elimination this alternative would seem to be responsible.

At present it seems only safe to conclude that some substance is produced by the heart or gills under the conditions of the perfusion which, when accumulated by repeat circulation, brings about early total failure of the heart.

Discussion of the secretory activity of the gills.

The results of the experiments in which the ventral aorta-gill preparation was perfused with repeat circulation answer the question asked on p. 226. Total osmotic concentration and chloride both decreased in approximately equal amount in osmotic terms during the passage through the gills bathed with sea water. The agreement between chloride and vapour-pressure determinations was within the experimental error in five cases, while only one of the seven experiments in this group yielded results in which there was a serious discrepancy. In this last case (experiment 6 B, Table I) vapour pressure increased to a considerably greater extent than was indicated by the chloride measurements, and repetitions of the analyses failed to reveal any error.

The mean concentration change, calculated as mg. chloride transferred from the internal to the external medium per hour per kg. of eel, was 8·2 from the chloride determinations and 7·4 from the vapour-pressure measurements. It should be remarked that the transformation of vapour pressure into mg. Cl^- assumes that chloride is accompanied by a monovalent cation. Omitting experiment 6 B, the mean changes are 8·6 and 6·2 from Cl^- and vapour pressure respectively.

Smith's [1930] investigation of water and salt balance in eels in sea water indicates that calcium can hardly be eliminated from the gills. In two of the present experiments measurements of calcium concentration were kindly made for us by Miss E. Watchorn; they indicated an increase of calcium content of the order of less than 1 p.c. of the initial calcium. It is clear that chloride was not eliminated in company with calcium, so that any chloride secreted must have been accompanied by sodium, potassium or hydrogen, the only other cations present.

There was an indication that the vapour-pressure change might be slightly less than that which would have been expected from the con-

centration change of the chloride. This, as well as the behaviour of the calcium concentration, is what would be expected if the changes are brought about by means of secretion of a concentrated chloride solution from the gills.

The results of these experiments are, then, in complete harmony with Keys' previous experiments with the double perfusion heart-gill preparation and with Homer Smith's conclusions with regard to the water and salt cycle in the normal intact marine teleost. Smith's position is clear: "...it is inferred that the marine fish excretes by some extrarenal route a solution of NaCl and KCl which is hypertonic to the ingested sea water and thus leaves part of the absorbed water free for the formation of the osmotically dilute urine and intestinal residue" [Smith, 1930, p. 502]. Smith's surmise that the gills may be involved in the extrarenal excretion has already been shown to be correct by the previous gill perfusion experiments; the present work reinforces the evidence.

Comparison may be made between these experiments and the Copenhagen experiments with the heart-gill preparation with regard to the net chloride secretion per hour per kg. of eel. In these terms the mean secretion observed here was roughly half that found in the previous work in which the same concentration of internal perfusion fluid was used. There are several possible explanations of this difference. The eels used in the experiments reported here were less healthy than those used in Copenhagen and, also, were seldom so well habituated to sea water before the experiment. Perhaps even more significant is the strong probability that toxic substances (metabolites?) are accumulated in the repeat circulation experiments. Single circulation experiments in this laboratory have indicated rates of chloride secretion similar to those obtained in Copenhagen, although low values were obtained occasionally.

The experiments with the repeat circulation heart-gill preparation indicated a decrease in chloride concentration of the internal medium similar in amount to that observed in the ventral aorta-gill experiments, but the vapour-pressure measurements clearly showed an increase in total osmotic concentration. The first possible explanation of this phenomenon which we considered was that the heart enriches the concentration of the perfusate by adding to it the products of the metabolic degradation of the glucose in the perfusion fluid. To cause the observed increase in osmotic concentration relative to the chloride concentration at least 50 p.c. of the sugar would have to be broken down to lactic acid and carbon dioxide. We found, however, that there was either no change

or a very slight increase in sugar (reducing substance) concentration after repeated circulation through the heart-gill preparation.

The behaviour of the heart in the repeat circulation experiments must be borne in mind. There was definite evidence that the heart was poisoned by some substance added to the circulating fluid from the tissues of the heart. If this hypothetical substance was also the cause of the increased osmotic concentration it must either be produced in relatively great quantity or be of small molecular weight. More probably the poisoning of the heart and the increase of osmotic concentration were brought about by different substances, but the alteration of permeability in the partially poisoned heart might bring about the osmotic effect.

One outstanding fact remains clear: the gills themselves effect a total concentration change opposed to the concentration gradient which is roughly equivalent to the change in chloride concentration if the chloride is reckoned as NaCl and KCl. All doubts as to the need for the expenditure of considerable amounts of energy by the gills bathed in sea water necessarily disappear.

Thermodynamic calculation of the minimum work involved in the gill secretion.

The estimation of the minimum thermodynamic work done in the secretory activity of the gill is made possible by the parallelism between chloride and total osmotic concentration. It is known that W, the work done in transferring 1 g. mol. of an ion, reversibly and isothermally, from a solution in which its concentration is C_i, to a more concentrated solution in which its concentration is C_e, is given by the formula:

$$W = 2{\cdot}303\, RT \log_{10} \frac{C_i \cdot \gamma_i}{C_e \cdot \gamma_e}, \qquad \ldots\ldots(1)$$

where $R = 1{\cdot}985$ in calories, T = absolute temperature, γ_i and γ_e are the activity coefficients of the ion in solutions of concentrations C_i and C_e respectively, all concentrations being expressed as g. mol. per 1000 g. water.

For a numerical calculation we may take data from Keys' paper [1931 *b*, p. 370]. The experiment dated April 20 indicated that the gills secreted 0·31 c.c. of a solution containing 52 g. Cl per litre in a period of 2·5 hours, that is 0·124 c.c. per hour of a solution 1·50 molar in Cl or 0·000186 g. mol. Cl^-. Transport of an equal amount of Na^+ (or K^+) must be assumed. The molar concentration of Cl (and Na) was 0·197 in the internal fluid and 0·537 in the external fluid, the corresponding activity coefficients being 0·752 and 0·689 [Lewis and Randall, 1923,

p. 351] respectively. The work done, then, in the transport of 0·000186 g. mol. of NaCl at 288° K. (the experimental temperature) was 0·195 cal.

In addition to the NaCl, water was transported across the gills, but as the vapour pressure of water was lower in the external fluid, the work involved in the transport of water must be subtracted from the work done in the transport of NaCl.

From the molar concentrations it is possible to calculate the weight and also the molar fraction of water, N_w, in both fluids. In the internal fluid

$$N_w = \frac{55 \cdot 2}{55 \cdot 2 + 0 \cdot 4} = 0 \cdot 993,$$

and in the external fluid

$$N_w = \frac{55 \cdot 0}{55 \cdot 0 + 1 \cdot 1} = 0 \cdot 980.$$

By applying formula (1) to the values of N_w on both sides of the membrane, it appears that the work done by the transport of 0·0065 mol. of water was 0·048 cal. The net work done in the secretion is therefore 0·195 – 0·048 = 0·147 cal. per hour.

The gill tissue in eels of this size has been weighed in a number of cases and it amounts quite constantly to about 5 g. per kg. of eel when weighed wet after dissecting free from the gill bars. The total body weight of the eel in the above experiment was 153 g.[1], and the wet weight of the gills may be put as 0·8 g., so that the work of secretion per g. of gill tissue per hour becomes 0·184 cal.

Comparison with data from other experiments indicates that the secretion work done in the experiment above, although not unusual, may be somewhat higher than normal for this concentration of internal fluid. The water transport term is open to some uncertainty owing to the difficulty of the volume measurements, although the calculation of the water movement from the total volumes and chloride contents of the two systems at the beginning and at the end of the experiment [Keys, 1931 *b*, equation I, p. 372, equation II, p. 373] agreed closely with the observed water movement. Making all due allowance for error, the minimum thermodynamic work required in the gill secretion appears to be between 0·1 and 0·3 cal. per hour per g. of gill tissue.

Similarities between the activity of the sea-water fish's gills and the mammalian kidney have already been pointed out by one of us (A. K.). It is of interest to compare the minimum thermodynamic work in the two cases. Borsook and Winegarden [1931] calculate the minimum secretion work of the "normal" human kidneys as 704 cal. per day.

[1] The weight "453" g. on p. 370 of Keys' paper is a misprint.

If we take the weight of the kidneys as 300 g. each, the work becomes 0·049 cal. per hour per g. of kidney tissue. The amount of concentration work of the perfused eel gill would appear to be somewhat greater than that of the normal human kidney when equivalent amounts of tissue are compared, although no emphasis should be placed on this difference. The amounts of work done in the two cases are certainly of the same order.

The most interesting result of the calculation of Borsook and Winegarden is the indication of the very low efficiency of the kidney. Taking the measurements of the oxygen consumption made by Barcroft and Straub [1910] the kidney secretion appears to be done with an efficiency of 1–2 p.c. Unfortunately there are as yet no data as to the efficiency of the eel gills. However, the possible cost of the gill secretion may be calculated in terms of oxygen consumption[1].

Taking a respiratory quotient of 0·80, the combustion of 1 c.c. of oxygen yields 4·8 cal., according to Lusk's [1924] revision of the table of Zuntz and Schumburg. The oxygen equivalent of the caloric requirement calculated above for the secretion is then

$$\frac{(0\cdot147)\,(1000)}{(4\cdot8)\,(153)} = 0\cdot200 \text{ c.c. of oxygen per kg. of eel per hour.}$$

On the generous assumption of an efficiency of 30 p.c. this becomes 0·67 c.c. O_2 per hour per kg., a not inconsiderable fraction of the total resting metabolism of the eel which Krogh [1904] found to be about 30 c.c. O_2 per kg. per hour at this temperature. If the efficiency of the secretion activity of the gills is no greater than that of the human kidney, the oxygen consumption required for the gills alone may be 15 times as great, or 10 c.c. per hour per kg. In other words the secretion activity of the gills of the eel in sea water may account for between 2 and 30 p.c. of the total resting metabolism of the animal when the concentration of the internal medium going to the gills is fairly high. It is of interest to note that Schlieper [1929] concluded from his oxygen consumption measurements that the cost of maintaining the normal concentration of the body fluids in some homoio-osmotic marine invertebrates such as *Carcinus* represents a considerable fraction of the total resting metabolism.

[1] While in all probability the energy used in the secretion of the chloride is derived from oxidative processes in the gills, it is recognized that this may not be the case, and accordingly these calculations do not necessarily relate to the oxygen consumption of the gills themselves.

SUMMARY.

Gill perfusion experiments involving repeated circulation were carried out on eels previously adapted to sea water. Apparatus and operative procedures are described for two types of preparation. Measurements were made of changes in the vapour pressure and the chloride concentration in the perfusion fluid circulating through the gills bathed externally in sea water. Observations were made of calcium and glucose concentrations in the perfusate and of œdema in the eel head. A thermodynamic calculation is made for the energy cost of the gill secretion. The principal results and conclusions are:

1. The failure of circulation when the gills are perfused from the ventral aorta under constant pressure is due to the constancy of the pressure; when pulsating pressure is supplied from an artificial heart satisfactory perfusion may be maintained at a constant rate for hours.

2. Under the conditions of repeated circulation of the heart-gill preparation the heart is gradually poisoned by some substance released from the tissues and accumulated in the perfusate.

3. Chloride secretion in opposition to the concentration gradient is shown in the repeat circulation heart-gill preparation, but the chloride concentration change is not paralleled by a similar change in total osmotic concentration. This discrepancy is believed to be related to the peculiarity of the eel heart under the conditions of repeated circulation.

4. The isolated gills in the ventral aorta-gill preparation with sea water outside bring about a decrease in the osmotic concentration of the internal medium, and this is explicable quantitatively by secretion of a concentrated chloride solution.

5. The greater part of the loss of perfusion fluid in the various preparations is due to filtration into the tissues of the head and is measurable as œdema.

6. The minimum energy cost of the gill secretion in the perfused gills bathed in sea water is of the order of 0·1 to 0·3 cal. per g. of gill tissue per hour, and this may represent a considerable fraction of the total resting energy expenditure of the eel.

7. In terms of g. of tissue the work done by the gills of the eel in maintaining the normal blood concentration in a sea water external environment is of a similar order to that done by the human kidney in its function of regulating the blood concentration.

8. The osmotic secretory activity of the gills is largely confined to the secretion of chloride and a monovalent cation, probably sodium, together with a small amount of water.

Our best thanks are due to Prof. Joseph Barcroft for his stimulating interest in this work.

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THE PORTAL CIRCULATION.

I. The action of adrenaline and pituitary pressor extract.

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For some time past the writer has been engaged in a study of the pathology of that ill-understood disease group commonly termed "splenic anæmia," which is often associated with gross liver disease and marked circulatory disturbances in the liver. In the spleen, appearances were frequently present which gave evidence of hæmorrhage at the point where the arterial capillaries of the spleen finally open into the pulp. Such hæmorrhage was found to be associated with marked pathological changes in the splenic vein, suggestive of long-continued intravascular strain on this vessel, and clinically the patients frequently vomited blood. The general conclusion was reached that these hæmorrhages in the spleen and stomach, with thickening of the splenic and portal veins, were the anatomical expression of increased pressure in the portal venous system [McMichael, 1931]. While the occurrence of such a raised pressure may be easily visualized in the presence of a shrunken and distorted liver, it was nevertheless obvious during this study that the changes described above occurred in the absence of gross cirrhosis. Indeed they were present in cases in which the liver, at the operation of splenectomy, appeared perfectly normal. It seemed clear then that the condition of "portal hypertension" which has been postulated to account for the appearances can occur in the absence of such gross and late changes as are found in a hob-nailed liver.

Even in the presence of gross cirrhosis the etiology of the raised pressure in the portal system is as yet imperfectly understood. Herrick [1907] observed that the portal cirrhotic liver gave passage to an amount of portal flow in proportion to its weight, and that there was no obstruction to the portal blood vessels from scar contraction in the large portal cirrhotic liver. On the other hand it was found that the portal pressure in cirrhosis was markedly influenced by the pressure at which fluid was passing into the liver through the hepatic artery. In the normal liver, alterations in the arterial pressure had little effect on the portal

pressure. In perfusion experiments on cirrhotic livers the addition of arterial inflow to the portal inflow markedly limited the latter, and for the portal flow to predominate, as in normal conditions, a portal pressure of 50 mm. Hg might be required. McIndoe [1928], on the other hand, was unable to confirm these observations of Herrick. In the cirrhotic livers which McIndoe studied, he found that the liver cells were practically divorced from their portal blood supply and that the vessels appeared to run in the connective tissue bands between the islets of surviving liver cells. Slow obliteration of the porto-hepatic venous communications might occur here by scar contraction.

Whatever the explanation of the portal hypertension in the late stage of the disease, and both authors may be partially correct at such a stage, it is quite clear that raised portal pressure occurring before "cirrhosis" has appeared cannot be dependent on scar contraction. If portal hypertension is present in the early stages of liver disease it must depend more on an alteration of physiological conditions of the portal circulation than on gross anatomical changes.

The approach to the problem of altered physiology in disease must be made through normal physiology, and here we find information on the normal circulatory conditions of the portal circulation deficient and inconclusive.

The portal circulation presents a problem both from the anatomical and physiological standpoints, and the conceptions of its intrahepatic anatomy must of necessity influence our interpretation of the vaso-motor reactions in this system. It is obvious that there must exist some mechanism whereby the pressure in the hepatic artery is reduced to a level equal to that in the portal system when the two blood streams meet and mix in the sinusoids of the liver lobule. Some of the earlier physiological observers, *e.g.* Burton Opitz [1912], and Macleod and Pearce [1914], seemed to accept the work of Gad [1873], who considered that the hepatic arterial and portal venous streams met by a vascular connection at an acute angle whereby a wedge-shaped flap valve was formed at the angle of union. This flap was said to shift in accordance with the pressure brought to bear on its sides, so that an increased flow of blood through the hepatic artery would limit the flow of blood through the portal vein.

This view, however, is not in keeping with the bulk of the anatomical evidence. Kiernan [1833] studied the intrahepatic distribution of the hepatic artery, and concluded that it subdivides into branches which supply the bile ducts, the walls of the portal veins and the tissues of the portal tracts (capsular branches, Fig. 1). From the capillary network thus formed, collecting venules gather the blood into the portal veins. These small intrahepatic radicles of the portal vein are termed internal hepatic radicles (Fig. 1). This work of Kiernan received authoritative support from the researches of Cohnheim and Litten [1876], Pfuhl [1922], Loeffler [1927], and Cameron and Mayes [1930]. Loeffler draws attention to the fact that the blood supply to the intrahepatic bile ducts corresponds in its anatomical arrangements to the vascular supply of the gall bladder, which develops as a diverticulum of the bile ducts.

Cameron and Mayes admit, however, that an occasional small branch of the hepatic artery may pass directly into the liver sinusoids, and Olds and Stafford [1930] maintain that such branches constitute the main mode of distribution of the hepatic artery. If such branches are given directly to the lobule they must be of the nature of very fine arterioles to achieve a reduction of the pressure in the hepatic artery to a level corresponding to that in the portal vein.

The prevailing views of the French workers in this field are summarized in Poirier and Charpy's treatise [1905]. While accepting Kiernan's views, occasional small branches are described which pass directly to the liver lobule. The capsular branches of the hepatic artery, after supplying the structures in the portal tracts, terminate not only in the internal

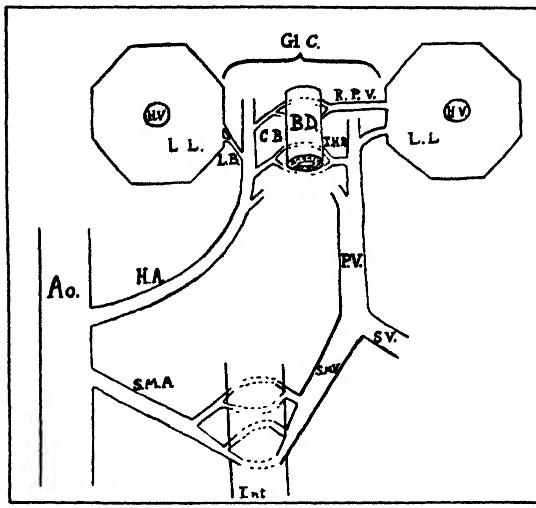

Fig. 1. Diagram of the hepatic and portal circulation. The dotted lines represent capillaries. Of the intrahepatic branches of the hepatic artery, the lobular branches are the least numerous and the smallest.

Ao. aorta. *B.D.* bile duct. *C.B.* capsular branches. *Gl.C.* Glisson's capsule. *H.A.* hepatic artery. *H.V.* hepatic vein. *I.H.R.* internal hepatic radicle of Kiernan. *Int.* intestine. *L.B.* lobular branch. *L.L.* liver lobule. *P.V.* portal vein. *R.P.V.* radicular portal vein (Ferrein). *S.M.A.* superior mesenteric artery. *S.M.V.* superior mesenteric vein. *S.V.* splenic vein.

hepatic radicles, but also link up with small veins which pass directly into the liver lobule without joining the portal vein. These small radicular portal veins were apparently discovered by Ferrein [1749].

These anatomical communications do not appear to have been considered in studies of the physiology of the portal blood flow. Clark [1928] and Bainbridge and Trevan [1917], for example, assumed that there were no true capillaries in the liver, while other writers do not commit themselves to any definite opinion.

The importance of keeping these anatomical considerations in mind cannot be over-emphasized in the study of the hepatic circulation. In

perfusion experiments on the isolated liver it is well known that, after about half an hour, the pressure in the portal vein must be raised to a level comparable to that in the hepatic artery, in order to maintain a flow through the organ. This suggests a free anastomosis between the two systems. This anastomosis may take place by three possible routes:

(*a*) *Via* the lobular arterioles through the sinusoids at the periphery of the lobule.

(*b*) *Via* the capsular branches through radicular portal veins (Ferrein), and the peripheral sinusoids of the lobule.

(*c*) *Via* the capsular branches through the internal hepatic radicles of the portal vein (Kiernan).

It is difficult to simplify this statement further for the purpose of physiological considerations. One fact, however, is of paramount importance: an arteriolar system is a theoretical necessity in order to reduce the great arterial pressure of the hepatic artery to a level equal to that in the portal vein, for these pressures must be equal when the two streams reach the hepatic sinusoids. The balance of anatomical evidence is in favour of a capillary communication between the hepatic artery and the portal vein. Whether the capillary communication is physiologically important will be considered in this paper.

Keeping this knowledge of the hepatic circulation in mind, we approach the problem of the portal circulation from a new aspect. We conceive of the portal venous system as having an outlet through the liver sinusoids and being fed with blood from the mesenteric arteries in the periphery, and also having a central inflow from the internal hepatic radicles inside the liver. This central inflow joins the portal ramifications immediately before the point at which the portal blood passes into the liver sinusoids. Just beyond this point the portal stream is joined by a further inflow from the lobular arterioles and the radicular portal veins.

The object of the work to be detailed in this paper is the elucidation of the effect of various vaso-motor reactions in the liver on the portal venous pressure, and if possible to form an estimate of the relative importance of the routes of anastomosis described between the hepatic artery and the portal vein.

Technique and methods.

Cats were used throughout the experimental work, anæsthetized with luminal (0·2 g. per kg. intramuscularly), or with chloralose (0·1 g. per kg. injected into the intestine after the cat had been anæsthetized with

ether). There was no difference in the arterial or venous blood-pressure reactions with these two drugs, but with the latter the arterial pressure was more satisfactorily maintained. The arterial pressure was recorded from the carotid artery in mm. Hg. The portal pressure was recorded from the stump of the splenic vein, the spleen having been removed. At first it was recorded with carbon tetrachloride in the manometer (S.G. 1·58). In the later experiments when technical difficulties regarding floats for the manometer had been overcome, a 5 p.c. solution of sodium citrate in water was used to fill the recording system. A very satisfactory water manometer for ordinary purposes was made by C. F. Palmer, Ltd., after the model suggested by Thompson [1930], for taking continuous blood-pressure records. A more delicate and accurate instrument was constructed for this work by Mr Fraser of the Natural Philosophy Department, Aberdeen University. Once the cannulæ were in position and the pens writing on the drum, the abdomen was closed as far as the requisite manipulations would permit. The component vessels of the portal circulation were rendered easily available by loops of thread passed round them, so that they could be clamped or otherwise obstructed at any time during the experiments.

The possible complicating influence of the return of blood from the stomach to the portal vein was excluded by ligaturing the gastro-duodenal branch of the hepatic artery. This procedure cut off the main blood supply to the stomach. The only other tributary of the portal vein outside the liver is the cystic vein; this is so small as to be practically negligible. In one critical experiment, however, the cystic vein, too, was ligatured.

For the liver-volume observations a plethysmograph of the type suggested by Griffith and Emery [1930] was used, connected to a McDowall volume recorder.

Component factors maintaining portal pressure.

In agreement with other investigators in this field, the blood-pressure in the portal system in cats was found to be on the average about 80–100 mm. water or 50–60 mm. carbon tetrachloride. The pressure undergoes fluctuations with respiratory movements.

It rises during inspiration and falls on expiration. According to Schmid [1909] these variations are due to changes in intra-abdominal pressure. They are not due to respiratory alterations in vena caval pressure near the heart, as these changes take place in the opposite direction, *i.e.* a rise during expiration and a fall on inspiration. The fluctuation is not due to the transmission of pressure from the arteries, for the respiratory change in pressure in the latter does not coincide absolutely with the respiratory movements, as the portal

pressure does. Since the fluctuations are present when the abdomen is open the intra-abdominal pressure cannot be entirely responsible for the change. When the portal vein was guarded against direct pressure from the movements of adjacent viscera, *e.g.* the duodenum, the respiratory fluctuations were still present. It would appear, therefore, that under the present experimental conditions the factor responsible for the respiratory fluctuations may possibly be the downward movement of the diaphragm. This would produce a simple mechanical effect, the descending midriff gently squeezing the liver and causing a temporary obstruction to the hepatic circulation.

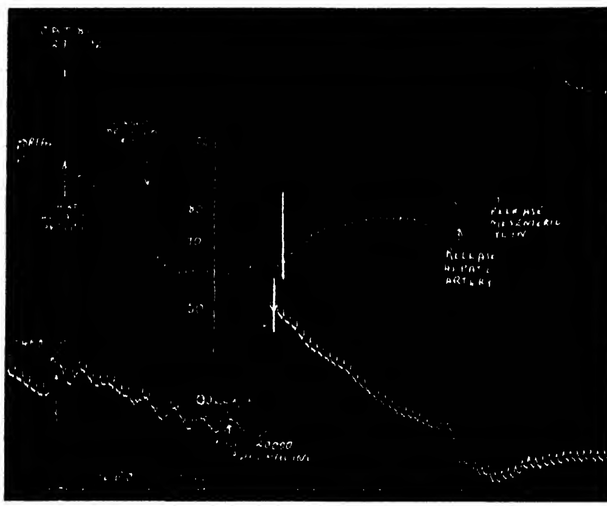

Fig. 2. Upper record: portal pressure. Lower record: arterial pressure. Chloralose anæsthesia. Hepatic artery clamped at the first arrow. This reduces the portal pressure by 15 mm. water. At the second arrow the mesenteric vein was clamped. The portal pressure is further reduced by 28 mm. water, but is still maintained at a relatively high level. The injection of adrenaline into the saphenous vein has no effect on the portal pressure until the arterial pressure has begun to fall. The slight rise in portal pressure is probably effected by increased inflow through collateral arterial channels from the bare area of the diaphragm. The removal of the clamps from the vessels allows the portal pressure to return to a high level.

By producing a simple obstruction of the various component vessels of the portal circuit various effects are obtained. Clamping the superior mesenteric artery or vein cuts off the splanchnic inflow and leads to an immediate fall in portal pressure by some 30–40 mm. water. The pressure, however, does not fall to zero and a pressure of 50 mm. water is maintained. As the only remaining inflow to the liver after this procedure is the hepatic artery it would appear that the latter pressure is maintained, in part at least, by a transmission of pressure from the hepatic artery to the portal vein (Figs. 2 and 5).

In comparison with the effect of clamping the mesenteric vessels, obstruction of the hepatic artery alone causes a slighter fall in portal pressure, usually amounting to 15–25 mm. water (Figs. 2 and 4). This is not surprising, for as Macleod and Pearce have shown, the hepatic artery brings about one-fourth to one-third of the blood which flows through the liver. Grab, Janssen and Rein [1929] estimate the proportion at much less—about one-sixth. Three-quarters or more of the blood flowing through the liver, therefore, comes from the mesenteric vessels. Obstruction of the hepatic artery thus only cuts off a minor part of the inflow and the pressure is well maintained, although at a slightly lower level, by the mesenteric portal flow. It appears from this that under normal conditions the main driving force of the portal system is *vis a tergo* from the mesenteric arteries. But since the pressure in the intact portal circuit is about 80 mm. water, and occlusion of both the hepatic and mesenteric components fails to reduce that pressure by more than half (Fig. 2), we must assume that there is a resistance to the portal flow beyond the point of entry of the internal hepatic radicles of the portal vein. This resistance may be offered in the sinusoid bed of the liver, but the degree of constriction of the portal ramifications in the liver before they enter the sinusoids, and of the sublobular venules, may influence the rate of escape of blood from the portal system. This hepatic resistance has the effect of reducing the portal pressure to the zero level which Bayliss and Starling [1894] recorded as being present at the point at which the hepatic veins join the vena cava.

THE EFFECTS OF ADRENALINE AND PITUITARY PRESSOR EXTRACT ON THE PORTAL BLOOD-PRESSURE.

I. *Adrenaline.*

This drug was used in quantities of 0·5 c.c. of a 1 in 10,000 or a 1 in 20,000 solution given intravenously. The former strength was used where the animals were anæsthetized with luminal, while the latter was given when chloralose was used. The injections were given into the saphenous vein usually, but for some special purposes they were made intraportally. The exact mode of administration is indicated on the tracings or in the legends.

By injecting adrenaline into a systemic vein a rise in portal pressure is produced. This begins about 7 sec. after the arterial pressure has started to rise, and it continues for several seconds after the arterial pressure has passed its summit (Fig. 3). The portal pressure rise has thus

two phases: the first occurring while the arterial pressure is still rising, and the second while the arterial pressure is falling. As will be shown later (Fig. 7), the rise in portal pressure after intraportal adrenaline begins immediately. We may assume therefore that the interval of 7 sec. between the onset of the rise of arterial pressure and that of the portal pressure is due to a delay in adrenaline reaching the portal system. It reaches the arteries before the veins and the effect of the drug is therefore first manifested on the arterial pressure record. The rise in pressure thus produced in the portal system may be due either to an increased inflow into the mesenteric or hepatic radicles of the portal vein, or to an obstruction to the outflow through the hepatic branches, or to a combina-

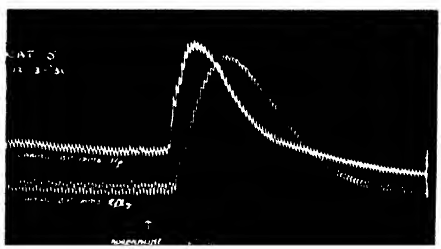

Fig. 3. Upper record: carotid pressure. Lower record: portal pressure (recorded in carbon tetrachloride). Luminal anæsthesia. At the arrow 1 c.c. 1/20,000 adrenaline injected into the saphenous vein. The carotid pressure begins to rise about seven seconds before the portal pressure. The portal pressure, however, continues to rise while the arterial pressure is falling.

tion of both. In comparison with alterations in inflow and outflow, constriction of the main trunk of the portal vein as a whole is not likely to occur to such a degree as to exert any marked effect on the portal pressure. This factor was ruled out by experiment (p. 254).

The mesenteric component.

If the portal vein is occluded partially by means of a ligature applied near the hilus of the liver, the portal outflow is so obstructed that the pressure sets itself at a higher level and alterations in pressure in the portal vein, dependent on intrahepatic effects of adrenaline, are prevented from manifesting themselves on the portal pressure record. By clamping the hepatic artery and thus preventing adrenaline from reaching the liver, the intrahepatic effects can also be abolished.

Under these circumstances the injection of adrenaline is followed by an immediate fall in portal pressure, which begins as soon as the arterial

pressure starts to rise. The moment that the arterial pressure passes its summit, however, the portal pressure begins to rise again and reaches its height about the time that the arterial pressure returns to normal (Fig. 4).

Thus when the hepatic component is excluded from the recording system the rise in portal pressure is delayed. Since the only factor which can influence the portal pressure from the peripheral mesenteric radicles is the inflow of blood into the system, we assume that the inflow through the mesenteric capillaries is first diminished and then increased. Clark

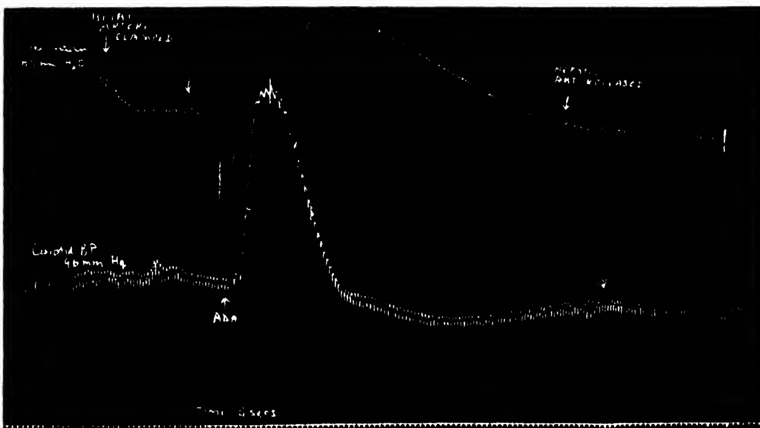

Fig. 4. Upper record: portal pressure. Lower record: arterial pressure. Luminal anæsthesia. At the first arrow the hepatic artery was clamped. At the second 0·5 c.c. 1/10,000 adrenaline was injected into the saphenous vein. As the arterial pressure rose the portal pressure fell. When the arterial pressure passed its peak, however, a rise in portal pressure began.

has measured the flow from the mesenteric veins under similar experimental conditions, and finds that under adrenaline the flow diminishes until the arterial pressure has passed its peak "when an obvious increase occurs which returns to the original rate as the pressure falls." The same observer states that "the fact that the outflow falls when the blood-pressure is at its highest indicates that the constriction of the intestinal vessels is sufficiently intense to prevent the high pressure forcing more blood through the vessels....This constriction, however, lasts a very short time and is rapidly overcome by the blood-pressure." These observations and views have been confirmed by the writer, using Clark's method of measuring the flow from a mesenteric vein by counting the drops. It

appears obvious, therefore, that during the initial stage the portal pressure falls from diminished inflow, due to the active constriction of the mesenteric arterioles. When this constriction passes off and the arterial pressure begins to fall there occurs an increased inflow of blood into the portal system. This increased inflow is facilitated by a dilatation of the arterioles and capillaries of the gut. Such capillary dilatation has been demonstrated by Clark, who considers it to be a passive phenomenon dependent on the increased pressure produced by adrenaline.

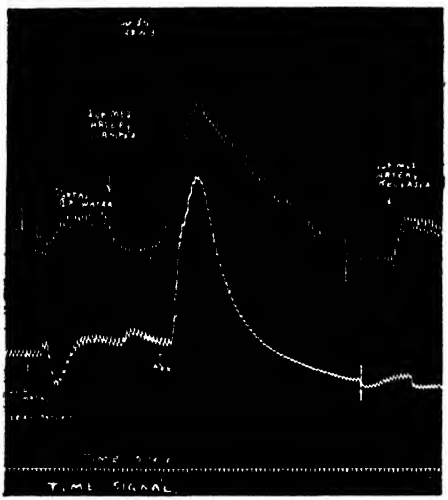

Fig. 5.

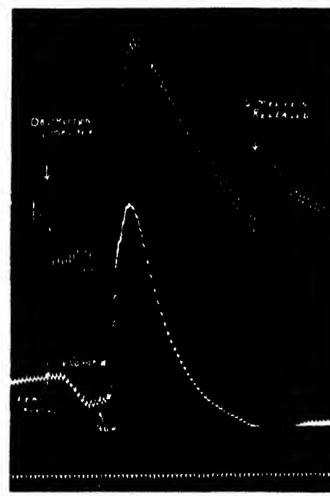

Fig. 6.

Fig. 5. Upper record: portal pressure. Lower record: carotid pressure. Luminal anæsthesia. At the first arrow the superior mesenteric artery was clamped. The injection of adrenaline into the saphenous vein then causes a rise in portal pressure beginning a few seconds after the arterial pressure has begun to rise. The portal pressure is still rising when the arterial pressure has begun to fall.

Fig. 6. Upper record: portal pressure. Lower record: carotid pressure. Luminal anæsthesia. The superior mesenteric vein was clamped at the first arrow. The injection of adrenaline into a systemic vein then causes the arterial pressure to rise, followed in a few seconds by a rise in portal pressure. The rise in portal pressure continues when the arterial pressure has passed its peak.

The hepatic component.

Having shown that the second phase of the adrenaline rise in portal pressure is due in the main to an increased inflow into the system from the dilating mesenteric arterioles, it may be assumed that the first phase is due to an intrahepatic effect. This seems to be the case. If we occlude

the return of blood from the mesenteric vessels, by clamping either the superior mesenteric artery or vein, the injection of adrenaline produces a rise of portal pressure which begins some 7 sec. after the arterial pressure has begun to rise. There is thus a short latent period exactly corresponding to that occurring with the portal circulation intact. The rise in portal pressure continues for some seconds after the arterial curve has passed its peak (Figs. 5 and 6).

This rise of pressure in the portal system occasioned by the action of adrenaline in the liver may result from any of the following causes:

(1) *A venous effect:* (*a*) By constriction of the intrahepatic ramifications of the portal vein beyond the point at which they receive the internal hepatic radicles.

(*b*) By constriction of the radicles of the hepatic vein.

(2) *A hepatic effect proper:* Obstruction of the sinusoids either by

(*a*) Swelling of the liver cells, or

(*b*) Constriction of the liver as a whole by contraction of plain muscle in the capsule of Glisson, narrowing the sinusoids.

(3) *An arterial effect:* By transmission of pressure from the hepatic artery into the portal vein.

The injection of adrenaline into the portal vein itself produces an immediate rise in portal pressure which precedes slightly the rise in arterial pressure (Fig. 7). The adrenaline reaches the liver before it reaches the systemic circulation and thus its hepatic effect appears slightly before the systemic effect. Having reached a certain level a small plateau is seen on the portal pressure curve, and a few seconds later, when the arterial pressure passes its peak, a secondary rise appears. Since this latter rise occurs at the same time as the rise resulting from an increased inflow through the mesenteric arterioles, it might be expected to be due to a similar effect, *i.e.* an increased inflow into the portal system through dilating arterioles. This is the explanation, but the arterioles are not those of the mesenteric, but those of the hepatic artery. This is shown by repeating the experiment with the hepatic artery occluded (Fig. 7), when it is seen that the initial hepatic rise still persists, but the secondary rise is almost negligible and is longer delayed than in the first experiment. It is noteworthy that the adrenaline effect on the systemic circulation after intraportal injection is much less than the effect produced by injection into a systemic vein. It would appear that the adrenaline is held up or altered in some way in the liver, so that a relatively smaller dose is passed on to the systemic circulation. In this way the mesenteric effect is also a minor one and only causes a slight rise in the portal pressure after the

arterial pressure has passed its peak. It is possible that this slight secondary rise may also be in part explained by a slight blood flow into the liver through collateral arterial channels from the bare area of the diaphragm (cf. Fig. 2). In any case the main fact remains that the occlusion of the hepatic artery abolishes almost completely the secondary rise in portal pressure following the intraportal injection of adrenaline.

That the hepatic artery plays an important rôle in producing the hepatic part of the rise of portal pressure after adrenaline is further

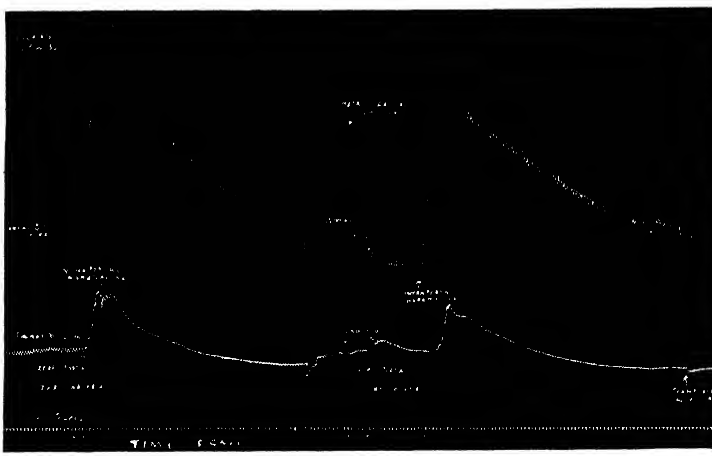

Fig. 7. Upper record: portal pressure. Lower record: arterial pressure. Luminal anæsthesia. At the first arrow 0·5 c.c. 1/10,000 adrenaline injected intraportally. The portal pressure rises before the arterial pressure, and a secondary rise of portal pressure begins as the arterial pressure passes its peak. At the second arrow the hepatic artery was clamped. The intraportal injection of adrenaline was repeated, but the secondary rise in portal pressure was abolished.

illustrated by the following experiments. If the mesenteric component is removed from the circuit by clamping the mesenteric vessels, and adrenaline is injected into a systemic vein, the portal venous pressure begins to rise, as we have seen previously (Fig. 6). While this rise is occurring, obstruction of the hepatic artery will produce an immediate trough in the curve of portal pressure. A very slight rise follows the trough which may be due to leakage through collaterals mentioned above (Fig. 8).

Fig. 9 shows the tracings of arterial and portal venous pressures obtained from a cat which had been subjected to removal of all the abdominal viscera with the exception of the liver. The portal pressure was

recorded by means of a cannula inserted into the stump of the portal vein. Under these circumstances the only inflow into the liver is through

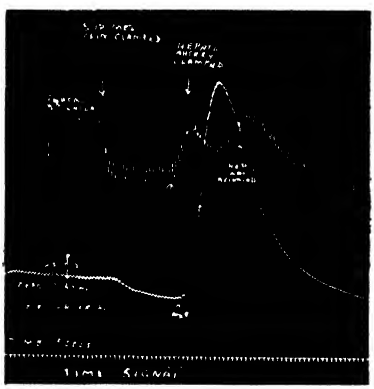

Fig. 8.

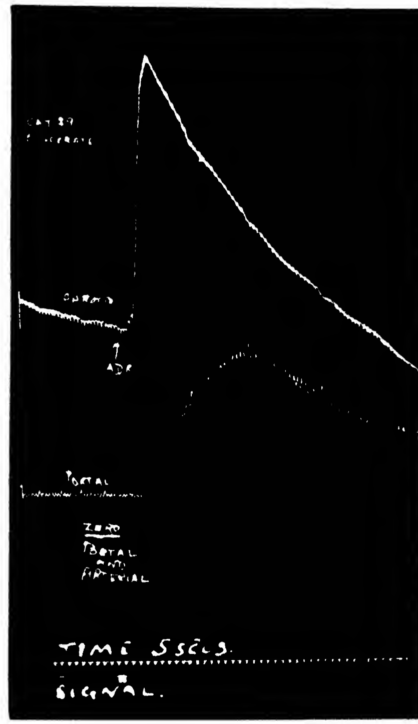

Fig. 9.

Fig. 8. (This record was taken from the same cat as Fig. 6.) Upper record: portal pressure. Lower record: arterial pressure. Luminal anæsthesia. The superior mesenteric vein was clamped at the first arrow. At the second arrow adrenaline was injected into the saphenous vein. During the first phase of the adrenaline rise in portal pressure, the hepatic artery was clamped. This procedure abolished all further rise in portal pressure for the time being, and the expected peak (cf. Fig. 6) was replaced by a trough.

Fig. 9. Upper record: carotid pressure. Lower record: portal pressure. Cat anæsthetized with luminal. In this cat the abdominal viscera had been removed with the exception of the liver. The injection of adrenaline into a systemic vein had no effect on the portal pressure until the arterial pressure had passed its peak. The rise in portal pressure then occurring is due to an increased inflow through the hepatic artery.

In this preparation the blood-pressure was falling rapidly, and the animal died a few minutes later.

the hepatic artery. The injection of adrenaline into a systemic vein has no effect on the portal pressure until the arterial pressure has passed its peak. At this point an immediate rise of portal pressure is seen. This rise

could only occur by a transmission of pressure from the hepatic artery into the portal vein by increased inflow through dilating arterioles. It is worthy of note that this preparation was failing, and it is probable that considerable vaso-constriction of the hepatic artery was present before adrenaline was injected. This would so limit the inflow to the liver that adrenaline would not reach the portal vein, and hence no initial rise in portal pressure would occur. The rise only began when the hepatic arterioles were dilating with the fall in arterial pressure. With marked constriction of the hepatic artery limiting the flow through it, the portal pressure was still maintained at 15–20 mm. water. This pressure must have been due to the resistance offered by the tone of the portal venous ramifications and the radicles of the hepatic vein. The reason for assuming pre-existing splanchnic vaso-constriction in the failing preparation will be elaborated later (p. 257).

The above facts present evidence which suggests very strongly that the second part of the hepatic component of the adrenaline rise in portal pressure is due to an increased inflow into the portal vein from the hepatic artery. How this increased inflow occurs, and the possible influence of intervening capillaries, will be discussed when we consider the action of pituitary pressor extract.

The first phase of the adrenaline rise in portal pressure.

The initial part of the adrenaline rise in portal pressure which occurs while the arterial pressure is rising is still unexplained. There can be no doubt that it is an immediate direct effect on the venous or sinusoidal bed of the liver, for it precedes the arterial effect after intraportal injection.

It is conceivable that the action of adrenaline in producing the initial rise in portal pressure after intraportal injection might be due to a diminution in the calibre of the main trunk of the portal vein itself. To test this possibility the portal vein was partly obstructed by a ligature so as to delay the entry of adrenaline into the liver after intraportal injection. This procedure also set the portal pressure at a high level which would not be influenced by any change in the calibre of the minute ramifications of the portal vein in the liver. Under these circumstances the intraportal injection of adrenaline was not followed by any rise in portal pressure. The injection of vasopressin under similar conditions produced a slight rise in portal pressure presumably due to contraction of the plain muscle in the wall of the vein. We assume, therefore, that with the doses of adrenaline used no appreciable effect is produced on the wall of the

portal vein so far as its main trunk is concerned. This, however, does not exclude an intrahepatic effect of adrenaline on the portal ramifications.

The only method by which the cause of the initial rise of portal pressure can be settled is that of plethysmography. The interpretation of the results of liver plethysmography has given rise to considerable difficulty and confusion in the past. Bainbridge and Trevan [1917] considered that the liver volume increased under the influence of adrenaline, and they ascribed this increase to a swelling of the liver cells. They stated that the action of adrenaline in causing a rise in portal blood-pressure could be simulated by the injection of distilled water into the portal vein. Distilled water would cause a swelling of the liver cells and thus the sinusoids would be obstructed. The writer has been unable to confirm this observation.

Clark [1928], on perfusing the excised liver, found that adrenaline caused a diminution of liver volume. Edmunds [1915] found that the action of adrenaline on the liver volume varied even in animals of the same species. The effect could be standardized to some extent by occluding the hepatic artery, after which adrenaline caused an increase in the volume of the liver. Edmunds is in favour of the view that adrenaline constricts the sublobular hepatic veins. François-Franck and Hallion, in 1896, observed that stimulation of the splanchnic nerves caused a diminution in liver volume even after ligature of the hepatic artery. This would suggest in contrast to Edmunds that the main site of action of adrenaline is on the portal venous ramifications.

During the past year valuable work has been done on this subject by Emery and Griffith [1930–31]. They present incontrovertible evidence that the hepatic nerves act on the portal ramifications. Adrenaline produces a diminution of liver volume similar to that occurring with stimulation of the splanchnic nerves. If the preparation was failing and the blood-pressure low, then the volume of the liver increased with adrenaline. Otherwise the initial effect of adrenaline was always a diminution in the volume of the liver.

This work carries conviction in that the experiments were carried out with the liver *in situ* in the anæsthetized animal, and thus the criticisms which can be directed against observations made on the excised organ are countered.

The writer has repeated the experiments of these observers so far as adrenaline is concerned, and finds himself in entire agreement with their main findings.

When adrenaline is injected into a systemic vein the liver volume diminishes markedly a few moments after the arterial pressure has begun to rise. As soon as the adrenaline effect begins to pass off the liver volume again increases (Fig. 10).

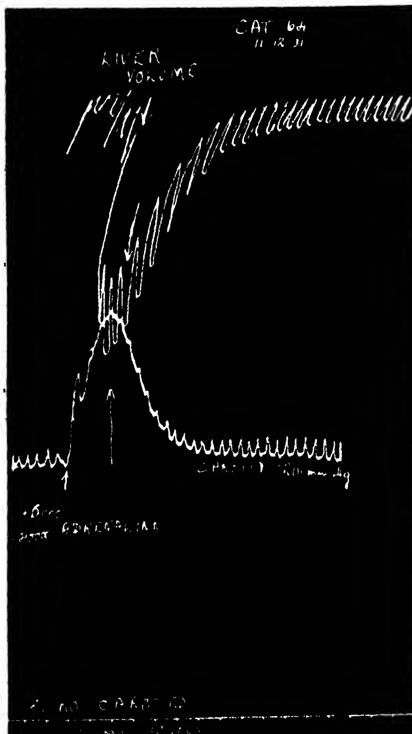

Fig. 10. Upper record: liver volume. Lower record: carotid pressure. Chloralose anæsthesia. Arrows mark corresponding times on the two curves. Following the injection of 0·5 c.c. 1/40,000 adrenaline into the saphenous vein the arterial pressure begins to rise, and almost immediately afterwards the liver volume diminishes markedly. The liver begins to swell again as the arterial pressure begins to fall.

It is obvious that an initial constrictor action of adrenaline on the sublobular hepatic veins would cause an increase in liver volume from retention of blood in the lobules. As this does not occur the initial venous effect of adrenaline must be on the portal ramifications. This view is confirmed by the fact that the intraportal injection of adrenaline brings about a diminution of liver volume before the adrenaline effect is manifested on the general circulation (Fig. 11).

When the preparation is failing, as Emery and Griffith have also noted, there is little or no alteration in the liver volume while the arterial pressure is rising after intravenous adrenaline. As soon as the pressure begins to fall, however, an increased inflow occurs through the dilating splanchnic arterioles and the liver volume increases. If a second injection of adrenaline be given at this time the volume of the liver diminishes with the rise in arterial pressure, and again increases as the pressure falls (Fig. 12).

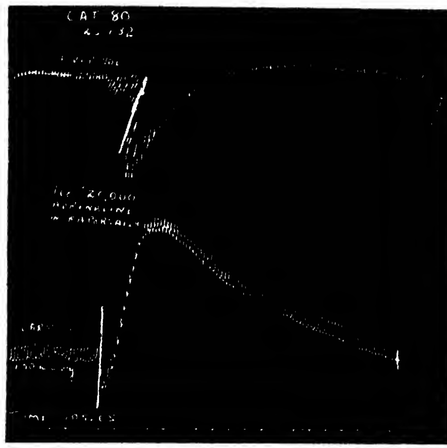

Fig. 11. Upper record: liver volume. Lower record: carotid pressure. Chloralose anæsthesia. The intraportal injection of 1 c.c. 1/20,000 adrenaline leads to an immediate diminution in liver volume. Some seconds later the carotid pressure begins to rise. The fall in liver volume is due to constriction of the ramifications of the portal vein.

Intraportal injection in the failing preparation gives a similar result. There is no effect on the liver volume until the splanchnic arterioles dilate, when an increase of volume occurs. A second intraportal injection then causes the liver volume to decrease.

These variations in the action of adrenaline on the liver volume require further elaboration. Reid Hunt [1918] made similar observations and noted that the animals which responded to adrenaline with an expansion of the liver appeared to be in a condition somewhat analogous to experimental shock. He says: "May not one of the features of experimental shock be a change in the blood-vessels of the liver such that they can no longer respond with a contraction to epinephrin...? The expansion of the liver after epinephrin appeared to be passive; it was accompanied by a marked contraction of the leg...."

The fact that no alteration takes place in the volume of the liver of the failing preparation until the splanchnic arterioles dilate may have two possible explanations:

(1) The liver sinusoids are already practically empty of blood, so that further constriction of the inflow cannot cause any diminution of the volume.

(2) The inflow to the liver is already so restricted by splanchnic vaso-constriction that adrenaline cannot cause any further vaso-constriction.

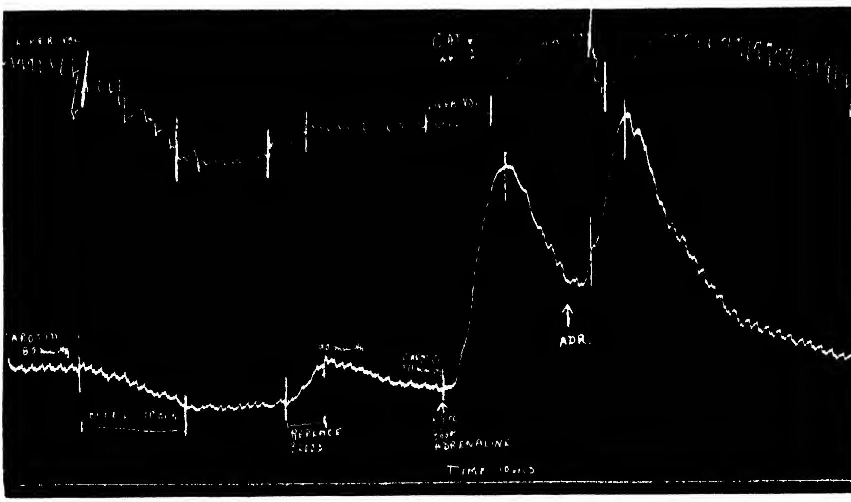

Fig. 12. Upper record: liver volume. Lower record: carotid pressure. Chloralose anæsthesia. Between the first two alignment marks 10 c.c. blood were withdrawn from the femoral artery into a heparinized syringe. Between the second pair of alignment marks this blood was reinjected into a saphenous vein. Subsequent injection of adrenaline into the saphenous vein caused no diminution in liver volume, but after the arterial pressure had begun to fall an increase in liver volume occurred. Note that the blood pressure was 70 mm. Hg when this injection of adrenaline was given. A second injection of adrenaline, however, caused a diminution in liver volume followed by an increase, as in the healthy preparation. Adrenaline fails to reduce the liver volume to the same degree as hæmorrhage.

The first hypothesis can be discounted, for we find that it is possible further to diminish the liver volume in such a failing preparation, *e.g.* by bleeding the animal. This is shown in Fig. 12. The second suggestion is probably nearer the truth, and is in accordance with Reid Hunt's theory. The record of portal pressure under the influence of adrenaline in a failing preparation shows neither the effect of diminished inflow

through the hepatic artery, nor any constrictor effect of adrenaline on the portal vein during the initial part of the rise in arterial pressure (Fig. 9). That the splanchnic vessels are not merely refractory to adrenaline is seen by their response to the second injection of adrenaline in Fig. 12. It would thus appear that considerable splanchnic vaso-constriction is present in a failing preparation. This may also explain the fact that although the arterial pressure may fall considerably from the level recorded at first, the portal pressure remains practically unaltered, for general splanchnic vaso-constriction would also cause constriction of the portal ramifications in the liver.

If this hypothesis is correct, it follows that the rise of blood-pressure from adrenaline in the animal with a low blood-pressure occurs by constriction of the blood vessels of the limb muscles and of the skin. This again is in accordance with Hunt's view. Vaso-constriction is already present in the splanchnic area and these vessels are only relaxed by the vaso-dilator impulses which bring about the fall in pressure after adrenaline.

The initial effect of adrenaline therefore is on the portal venous ramifications, where it causes veno-constriction. In the failing preparation this action may be masked by pre-existing vaso-constriction in the splanchnic area.

II. *The action of pituitary pressor extract.*

Vasopressin, injected into the systemic veins, produces a fall in portal pressure. This effect is due to the fact that vasopressin acts mainly on the capillaries and increases their tone [Clark, 1930; Krogh, 1929; Carrier, 1922]. As a result of this increase in capillary tone, the inflow of blood into the portal system is diminished and the pressure falls (Fig. 13). As Clark has noted, sometimes the portal pressure fails to recover its previous height after the injection of vasopressin. This effect is clearly shown in Fig. 14. Clark ascribes this to the fact that during the intra-abdominal manipulations there has been some loss of capillary tone which is restored by the injection of vasopressin and which remains after the effect of vasopressin on the arterial pressure has passed off. The action of the first dose is always much more marked than that of subsequent doses. When the mesenteric vessels are excluded from the portal circuit, the injection of vasopressin produces a further fall in portal pressure exactly comparable to that occurring with the portal circulation intact (Fig. 15). As this observation is exceedingly important, special care was taken to exclude all extra-hepatic communications between the hepatic artery and

the portal vein, by ligaturing the cystic vessels from the gall bladder and the coronary vein from the stomach.

This record is in marked contrast to the action of adrenaline under similar circumstances. As the arterial pressure passes its peak there is no rise of portal pressure comparable to that occurring with adrenaline.

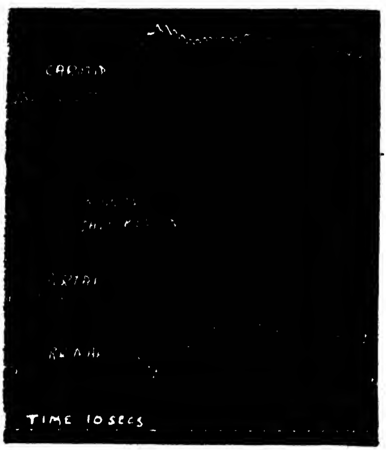

Fig. 13.

Fig. 14.

Fig. 13. Upper record: carotid pressure. Middle record: portal pressure. Lower record: vena caval pressure recorded from the renal vein. Chloralose anæsthesia. The injection of vasopressin into the saphenous vein leads to a rise in arterial pressure, accompanied by a simultaneous fall in portal pressure. The vena caval pressure remains practically unchanged.

Fig. 14. Upper record: arterial pressure. Lower record: portal pressure. Luminal anæsthesia. The injection of vasopressin into the saphenous vein leads to a fall in portal pressure occurring simultaneously with the rise in arterial pressure. The portal pressure fails to recover its original level as the arterial pressure falls again.

The action of vasopressin on the communication between the hepatic artery and the portal vein is exactly similar to the action of the drug *via* the mesenteric vessels. Therefore there must exist a capillary system between the hepatic artery and the portal vein inside the liver. It is unlikely that the liver sinusoids, with their incomplete lining, constitute a capillary system reacting like that in the intestinal wall. From this experiment we must assume that there is a closed capillary com-

munication between the hepatic artery and the portal vein in the portal tracts.

This assumption is further supported by the experiment shown in Fig. 8. During the venous part of the adrenaline rise of portal pressure, obstruction of the hepatic artery immediately abolishes any further rise

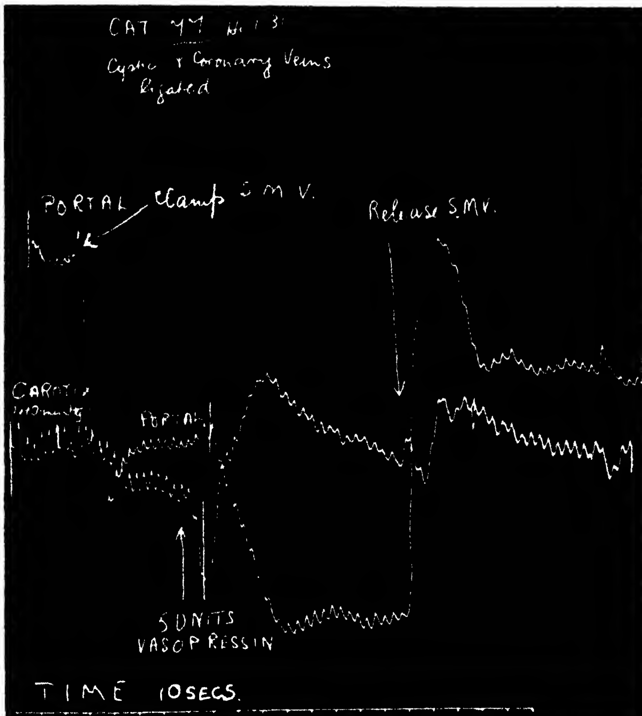

Fig. 15. Upper record: portal pressure. Lower record: carotid pressure. Chloralose anæsthesia. In this experiment special care was taken to ensure that there was no possible extrahepatic inflow into the portal vein, by ligaturing the cystic and coronary veins. At the first arrow the superior mesenteric vein was clamped. The injection of vasopressin into the saphenous vein then produced a very striking fall in portal pressure. On releasing the mesenteric vein the portal pressure did not return to its original level.

for the time being. If the communication between the hepatic artery and the portal vein was *via* the sinusoids at the periphery of the lobule, no such effect would take place. The ramifications of the portal vein are constricted before their entry into the sinusoids. Limitation of arterial inflow into the sinusoids would therefore have no influence on the rising

portal pressure. There must be a communication between the hepatic artery and the portal vein before the portal branches turn into the sinusoids.

The difference in the effects of vasopressin and adrenaline is due to a difference of the action of the drugs on the arteriolar and capillary systems. When the splanchnic arterioles relax after adrenaline the blood-pressure is able to force blood through the capillaries, which may either dilate passively before the increased delivery of blood through the dilated arterioles, which may conceivably undergo an active dilatation comparable to that observed by Hartman and his colleagues [1929] in the capillaries of muscle when adrenaline is applied to them directly. In the case of vasopressin, since the capillaries are actively constricted by the drug, the arterial pressure is prevented from having any appreciable effect on the portal pressure.

Summary.

1. The portal venous pressure in cats averages about 80–100 mm. water. It is maintained partly by the inflow through the mesenteric and hepatic arteries, and partly by a certain amount of tone at the outlet from the portal system.

2. These various components play a part in the vaso-motor reactions occurring under the influence of adrenaline and vasopressin.

3. Adrenaline causes first a vaso-constriction of the ramifications of the portal vein in the liver, leading to a rise in portal pressure. A secondary rise occurs from an increased inflow of blood into the portal system through the hepatic and mesenteric arteries.

4. The initial veno-constrictor effect of adrenaline may not be seen in the failing preparation.

5. Vasopressin causes a fall in portal pressure by producing constriction of capillaries, the inflow into the portal vein being thus diminished. This effect is the same whether the drug is acting *via* the mesenteric or hepatic arteries.

6. The vaso-motor reactions occurring between the hepatic artery and the portal vein in the liver are in all respects identical with the reactions occurring between the mesenteric artery and the portal vein. Indirect evidence suggests that the hepatic artery and portal vein are linked in the portal tracts by an arteriolar and capillary system.

In conclusion I have to express my indebtedness to Prof. J. J. R. Macleod, Dr J. W. McNee and Mr J. M. Peterson, for laboratory

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THE INFLUENCE OF SHORTENING ON THE HEAT PRODUCTION OF THE FROG'S GASTROCNEMIUS.

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THROUGH the studies of Fenn [1923, 1924] it is known that the performance of work by striated muscle is associated with the mobilization of energy in excess of that produced under isometric conditions, generally corresponding in amount to the work done. (See also Hartree and Hill [1928] and Hill [1930].) This general conclusion has been confirmed through measurements of the oxygen consumption in striated muscle by Fischer [1931] and in cardiac muscle by Stella [1931]. Rothschild [1930] came to the opposite conclusion as the result of lactic acid determinations made on semi-membranosus and gastrocnemius muscles of the frog. In these experiments much smaller quantities of lactic acid, amounting to 20–40 p.c. of the isometric value, were found when the muscle was allowed to shorten and do work. The reason for the discrepancy in the two sets of observations has been cleared up by the discovery of Hill [1930], through the method of myothermic measurements, that either result may be obtained in both sartorius and semi-membranosus muscles, depending upon the load, and consequently the length, under which the muscle shortens—the isometric energy is greater at the smaller loads, the isotonic at the heavier loads. In the case of the small parallel-fibred sartorius the Fenn effect is most readily obtained, while in the case of the stronger semi-membranosus the Rothschild finding occurs up to fairly heavy loads and therefore is the more commonly observed. The same relationship is indicated in the later experiments of Fischer and Stella. The gastrocnemius is not well suited to myothermic measurements by the ordinary methods on account of its large irregular form, and hitherto it has not been employed for the purpose of studying the relationship between the heat production and the accomplishment of work. The recent development of the single junction technique by Hill

[1931] has made possible the accurate measurement of the heat production of the gastrocnemius, and it is the purpose of the present paper to present some preliminary results obtained by this method.

Method.

The observations were all made on isolated gastrocnemii of large Hungarian *R. esc.* or English *R. temp.* Stimulation was secured through condenser discharges applied to the sciatic nerve. The myothermic technique was carried out exactly as described by Hill [1931], consisting essentially of a single iron-constantan junction inserted into the centre of the muscle, which indicated temperature changes through a sensitive Downing galvanometer. Not only does this method permit of the accurate measurement of the initial heat production in an irregularly shaped muscle, such as the gastrocnemius, but has for the present purpose the added advantage that the heat recorded is independent of the length of the muscle and so makes unnecessary calibration at various lengths. Further, the contact of the thermocouple with the muscle remains constant even when the latter is allowed to relax without a load, which makes possible the measurement of the heat production of the muscle while in a completely collapsed state. In carrying out an experiment, the usual procedure has been to place a certain load on the muscle and measure the initial heat production of a series of three isotonic twitches separated by intervals of 1 sec. After an interval of 5 min., during which the load was removed from the muscle, the observation was repeated with a stop against the muscle lever to give isometric contractions at the same muscle length as that for the isotonic contractions. In this manner pairs of observations were made in each preparation for various degrees of stretch and the results for the isotonic heat expressed as a percentage of the isometric heat at each muscle length. As a routine procedure the absolute values for the heat production (initial) were also determined according to the method described in detail by Hill [1931].

Results.

Since certain minor species differences have been observed in the course of the present study, the experimental data will be presented in two groups. The first series of experiments was made on the muscles of large Hungarian *R. esc.* These animals had reached the laboratory about 3 months previously and showed some muscle atrophy, as indicated by

an increase in the ratio of the length to weight as compared to muscles from freshly caught frogs. A tabulation of the essential data from six experiments is given in Table I. The exact meaning of the side headings

TABLE I. The influence of shortening on the heat production in muscles of *R. esc.*

Experiment	10. viii. 31		15. viii. 31		18. viii. 31		
	a	*b*	*a*	*b*	*a*	*b*	Average
Weight (g.)	—	0·445	0·812	0·779	0·400	0 370	0·561
Length (mm.)	—	36·0	40·5	40·5	35·5	35·5	37·6
Isotonic heat (p c.)	77	81	62	62	62	62	68
Isometric heat (200 g. p.c.)	—	—	89	83	88	83	86
,, (400 g. p.c.)	—	72	70	68	—	74	71
Maximum Fenn effect (p.c.)	11	23	25	11	6	1	13
Occurring at length (mm.)	—	41·5	44·6	44·8	38·7	37·6	41·4
,, load (g.)	200	338	320	331	197	200	263
Control isometric (p.c.)	—	72	71	83	88	91	81
Stretch (mm.)	2·2	2·2	1·3	1·8	1·3	1·6	1·6

requires explanation. The weight of the muscle represents the value found at the completion of the experiment after separating its attachment to the bones and cutting the tendon at its junction with the muscle substance. The "standard" length of the muscle was determined before removal from its mounting and refers to the portion weighed while under a slight stretch, equal to that produced by a load of approximately 15 g. which experience has shown to give a maximum response in the tension-length diagram. The values given for the isotonic heat on the next line were obtained with approximately this load and are expressed as a percentage of the isometric heat recorded for the muscle at the corresponding length. The value of the isotonic heat at this length (and small load) is always considerably under that of the isometric heat, averaging 32 p.c. less for the six experiments, and this agrees with the effect of shortening on the lactic acid production reported for this muscle by Rothschild.

In the case of the last two experiments of this series additional measurements of the heat production were made after removing all tension from the muscle and allowing it to rest in a collapsed state such that, even during contraction, no tension was transmitted to the lever; it was then found that the energy liberated fell to a very low value indeed. Under such conditions it is not possible to compare the heat with that produced isometrically at the same length, but in these two instances with repeated measurements the isotonic heats were, respectively, only 15 and 16 p.c. of the isometric heat at the standard resting lengths.

The fourth and fifth rows of Table I show the effect of increasing length on the heat production of the isometric twitch. These figures are ex-

pressed as a percentage of the isometric heat at the standard length; at lengths corresponding to loads of 200 and 400 g. the average values are 86 and 71 p.c. respectively. This decline in the value of the isometric heat, occurring from the beginning with increasing lengths, was a regular feature of this group of experiments. In part it can be accounted for by progressive changes, in the nature of fatigue developing in the course of the experiment. That this is not the whole explanation, however, is shown in Table I by the last row but one, which represent controls taken at the end of the experiment, *i.e.* after returning the muscle to the length existing under the small load initially employed of approximately 15 g. Under these conditions the heat again increased. It did not, however, reach the original value of 100 p.c. but on the average only 81 p.c. This may be due in part to the fact that under the heavy weights employed the loaded muscle was irreversibly stretched and at the end of the experiment was longer than at the start by an amount given in the last row.

As the length of the muscle was increased smaller isometric heats were recorded; the isotonic heats, however, became greater and, with sufficient loads, exceeded the isometric heats. This is the well-known Fenn effect. It reaches a maximum value, given in the table (as a p.c. excess of isotonic over isometric) along with the load and length at which it was obtained, and then declines when the load becomes excessive and the muscle shortens less during contraction. In this group of experiments the maximum value for the excess of isotonic over isometric heat averaged 13 p.c. and occurred at a length corresponding to a load of 263 g. The results from a typical experiment, 15. viii. 31*b*, are plotted in Fig. 1, in which the trend of the isometric and isotonic heats in relation to muscle length and load is clearly shown.

A second series of five experiments was carried out with the same technique but on muscles from freshly caught English *R. temp.* The results, summarized in Table II, are essentially similar to those obtained from *R. esc.* The isotonic heat is given as a percentage of the isometric heat throughout the range studied. Excluding the 7 g. load initially present, weights were added in increments of 35 g.: this caused a fairly uniform increase in muscle length; the figures given, therefore, for length are limited to the average values for the five experiments.

In this series, as in the previous one, the total energy liberated when the muscle shortened without producing tension was but a small fraction of the isometric heat at the standard length (7 g. load), averaging 23 p.c. in the five experiments. As the load (and length) is increased the isotonic heat increases in relation to the isometric heat and finally exceeds it. The

maximum excess is reached in the neighbourhood of 250 g., where the Fenn effect is on the average about 10 p.c. With greater loads the iso-

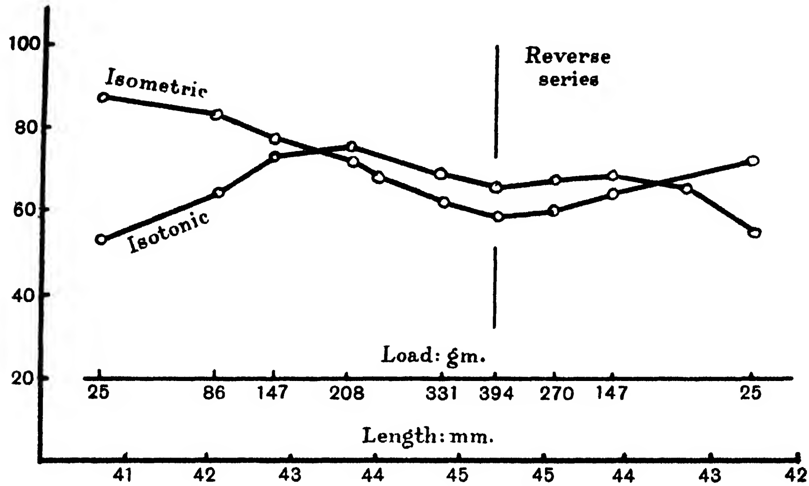

Fig. 1. Exp. 15. viii. 31. Gastrocnemius of *R. esc.* Influence of muscle length on initial heat production in isometric and isotonic twitches.

Table II. The influence of shortening on the heat production in muscles of *R. temp.*

Experiment	...	22. viii. 31	24. viii. 31	25. viii. 31 *a*	25. viii. 31 *b*	27. viii. 31	Average
Muscle weight (g.)	...	0·570	0·505	0·601	0·556	0·542	0·555
Load (g.)	Average length (mm.)	Isotonic heat (p.c.)					
0	27·5	25	19	25	24	22	23
7	32·5	51	46	52	46	50	49
35	33·9	59	60	61	—	—	60
70	34·8	68	70	—	[80]	67	68
105	35·0	74	79	76	—	—	76
140	35·5	89	—	—	89	91	90
175	35·7	98	98	93	—	—	96
210	36·1	102	—	—	100	101	101
245	36·5	—	108	105	—	—	106
280	36·7	108	—	—	108	110	109
315	37·2	103	98	100	—	101	100
7	33·5	49	51	54	50	49	51
0	27·5	28	17	23	—	20	22

tonic heat again becomes relatively less. A noteworthy point in Table II is the uniformity exhibited by the five muscles of the series, in the ratio between isotonic and isometric heat for any given load. Further, the controls, for both zero and 7 g. loads, obtained at the end of each experi-

ment when a certain amount of fatigue was evident, are in close agreement with the initial values. (Cf. the first and last two rows of Table II.)

There is one point, not shown in the tables, in which the data with *R. esc.* differ from those obtained with *R. temp.* With the latter the isometric heats were well maintained with increasing stretch, and in two experiments continued to become greater up to a length given by a tension of about 150 g. The isotonic heat values, however, increased also and at a greater rate, so that there is no significant difference between the two series in the general relationship of the isometric and isotonic heats with increasing muscle length. In the range of loads in which there was an excess heat production on shortening (Fenn effect) the isometric heat had, in every experiment, fallen, and in no instance did the value of the isotonic heat exceed the maximum for the isometric heat, which occurred at a shorter muscle length.

Discussion.

In his study of the relationship between the degree of shortening and the heat production at various lengths in the sartorius and semi-membranosus muscles, Hill [1930] presents strong reasons for believing that both the work done and the muscle length are important factors in determining the energy liberated. The present experiments on the gastrocnemius essentially confirm Hill's findings. The magnitude of the Fenn effect is, however, very much less than he reported; this appears to be related to the fact that in the present experiments, in contrast to those of Hill, the isotonic heat, even with the most favourable loads, never rose significantly above the maximum value of the isometric heat. This by itself would suggest that the energy liberated might be determined solely by the length of the muscle fibres, not only initially but during the course of the contraction. It is, however, quite improbable that there should be such a fundamental difference of response between the fibres in different muscles. In the case of the gastrocnemius the muscle is composed of relatively short, diagonally arranged fibres which are attached to a tendinous structure extending through a large part of the muscle. The result is a very inextensible muscle and the weights applied are largely supported by the connective tissue. It seems, then, extremely likely that the difficulty of obtaining the Fenn effect in the gastrocnemius is due to a failure to stretch the muscle fibres to the extent occurring, for example, in the sartorius.

As already pointed out, the measurements of the isometric heat in *R. esc.* always showed a decrease as the muscle length was increased:

in this respect the results accord with the earlier observations made on the sartorius (see Hill, 1925) but differ from those obtained in the present experiments with *R. temp.* in that the isometric heats were well maintained at increasing stretches. This difference might have its explanation in the fact that the Hungarian frogs had been in captivity for some months, unlike the English frogs which were freshly caught. It may, however, represent a true species difference, as has been observed in other experiments with these frogs (see Feng, 1932, and Cattell, Feng, Hartree, Hill, and Parkinson, 1931).

Of particular interest is the very low value to which the heat production falls when no work is done or tension developed. It has an obvious bearing on the economy with which muscle tonus may be maintained, for under these conditions it is clear that new fibres coming successively into activity must expend considerably less energy than under conditions in which they shorten against a load. From a technical standpoint, in connection with chemical procedures, it appears that if a muscle is frozen while in a relaxed condition changes brought about by the accompanying contraction will be minimized.

Summary.

1. Measurements of the initial heat production of the gastrocnemius of the frog show that with small loads isotonic twitches liberate less heat than isometric twitches at the same initial length. With greater loads the isotonic heat exceeds the isometric heat and a true Fenn effect is observed. These results accord with those previously reported by Hill for sartorius and semi-membranosus muscles.

2. In the case of muscles from *R. temp.* the heat in isometric twitches increased as the initial tension was raised and reached a maximum at a length given by a load of about 150 g. In muscles from *R. esc.*, on the other hand, the heat decreased from the beginning with increasing initial tensions.

3. When the gastrocnemius is stimulated in a completely relaxed state and produces no tension or work, the initial heat production is extremely small, amounting to less than one-fourth of the isometric value measured at the "standard" length of the muscle under a small initial load.

The author is greatly indebted to Prof. A. V. Hill, who suggested the problem and provided the facilities for carrying out the experiments.

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THE ANALYSIS OF THE DELAYED HEAT PRODUCTION OF MUSCLE.

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In earlier experiments on the analysis of the recovery heat [*e.g.* Hartree and Hill, 1922, 1924; Hartree and Liljestrand, 1926] the time of stimulus had to be short, 0·5 sec. or less, on account of the methods then used. The thermopile was purposely a slowly acting one, because the effect of the recovery heat on the galvanometer deflection was assumed to be nearly enough determined simply by subtracting from the "live" curve a "heating control" curve of the same maximum. It was supposed that the initial heat was so concentrated in time that the control curve gave a close enough determination of its effect at later times: this could be the case only if the heat was correctly "integrated," *i.e.* if the maximum deflection was independent of the distribution of the initial heat. Even if the defect in integration was only a small fraction, such a fraction of the control curve, especially when its height was still large, might be a serious part of the difference between "live" and "control."

The error can be avoided to some extent by taking the time of heating for the control curve about the same as the time of stimulus, but since the initial heat is by no means uniformly distributed during the time of stimulus, unknown errors are likely to arise for long stimuli. Further, since the galvanometer reached its maximum deflection in about 5 sec. and the "live" and "control" curves were then made equal in height and subtracted, no information could possibly be derived of the recovery heat during the first 5 sec. after the stimulus, and the next one or two steps of 5 sec. in the analysis were open to doubt. It had to be assumed, moreover, that any possible recovery before 5 sec. did not affect the maximum. Thus the curves showing the rate of recovery heat were not well determined near the start and not determined at all before 5 sec. These objections, it is admitted, would have little effect on the subsequent course of the analysis.

It is not possible by resources at present available to push much further the analysis of the initial heat [Hartree, 1931]. It was desirable, however, to use these resources in order to make a more accurate analysis of the course of the recovery heat, and in particular (1) to avoid the possible occurrence of the errors mentioned above, (2) to find the recovery heat starting immediately after the stimulus, and (3) to examine the conditions for any duration of stimulus.

PROCEDURE.

In recent experiments the following method was used. A fairly rapid thermopile, constructed by Mr A. C. Downing (constantan-iron couples, bakelite insulation, brass frame, glass cover), was connected with the very rapid moving magnet galvanometer described in the previous paper [Hartree, 1931]. Photographic records were taken on a quickly running drum so that the initial parts of the "live" curve could be analysed with sufficient accuracy. The heating control curves were usually for 0·1 sec., and the analysis of the initial heat was carried out with intervals of 0·1 sec. for short stimuli (up to 0·5 sec.), increasing to 0·4 sec. for long stimuli (up to 5 sec.). The photographs were continued on the drum for 12 to 15 revolutions (32–40 sec.), when successive rings came so close that there might be confusion if they were continued: after that the light was allowed to fall on the paper only for a fraction of a second, at intervals of 4 sec., up to 80 sec. or longer.

The analysis of the initial heat was usually carried to 4 sec. for a short stimulus and to 8 sec. for a long one.

From the results the heat, over the period for which the analysis was carried out, was distributed into equivalent amounts at equal intervals, usually 0·4 sec. The deflections due to these amounts at these times were then calculated for subsequent times from the control curve, which must therefore be determined at every 0·4 sec. over the whole time for which the recovery is to be calculated. The sum of such deflections at the subsequent times evidently gives the part of the observed deflection in the "live" curve which is due to the initial heat, or rather to the heat which has been included in the initial analysis, whatever its nature may be. (It will be seen later that a considerable part of the heat soon after the end of the long stimulus is not what is usually called initial heat.) This sum at subsequent times is then deducted from the live curve at the corresponding times, the difference being the deflection due to the heat which has occurred after the period of the analysis.

There is no chance of an error arising from a false maximum, as in the original method referred to above. With the thermopile used the deflection fell off rather quickly after its maximum. So far was it from "integrating" the heat from a long stimulus that the true heat was actually 25–30 p.c. more than that calculated simply from the maximum deflection. By the method described, however, the whole heat is properly taken into account[1].

Having obtained the deflections due to the heat occurring after the above analysis, these are in turn analysed to give the time course of the subsequent heat. The heat rate, except quite soon after a long stimulus, will now be very small compared with the initial heat rate, so it will be sufficient to analyse it in much longer time intervals: a suitable interval to use is 4 sec. Consequently the above calculation for the deflection due to the initial heat at subsequent times, and the measurements of the deflections from the live curve, are only required at every 4 sec.

It is impossible to analyse the "subsequent" heat directly by the control curve for 0·1 sec. heating, since this curve will lose a considerable part of its maximum deflection in 4 sec. There are two possible methods of procedure: (*a*) to make separate 4 sec. heating curves and to use them to analyse the subsequent heat in 4 sec. steps: the heat units, however, for the "initial" and the "subsequent" analyses will now be different and the "subsequent" analysis can only be expressed in terms of the initial heat (as is required) by making a still further analysis, namely, of the 4 sec. heating curve by the 0·1 sec. control: the further analysis could of course be avoided if the ratio of the heats given to produce the two control curves were accurately known; (*b*) a much better method is to "build up" a 4 sec. heating curve from the 0·1 sec. heating curve: this is very easily done since the ordinates of the latter must be found in any case at short intervals of time, as they are required for the initial analysis and for the calculation of the effect of the initial heat at subsequent times, as described above.

Besides saving time in taking and measuring further records, method (*b*) has the great advantage that the "built up" 4 sec. heating curve can be made for exactly the same heat as was used for the 0·1 sec. control, and the analysis of the subsequent part by this control will therefore be in the same units as those in which the initial heat is expressed.

[1] The rapid heat loss inevitably leads to the subsequent deflection being considerably smaller than for the slow-acting thermopile, with some loss of accuracy on account of this smallness. The method, therefore, is hardly suitable for investigating the total recovery heat.

Results.

The general form of the results of the original experiments was confirmed, the longer stimulus giving a larger and earlier maximum rate of recovery, stimuli of 4 or 5 sec. showing a comparatively enormous heat rate quite soon after relaxation. This rate, however, usually fell rapidly for about 15 sec. after which there was a distinct rise to a maximum at 25 to 30 sec., and then there was a gradual fall in the rate, as shown in Fig. 1.

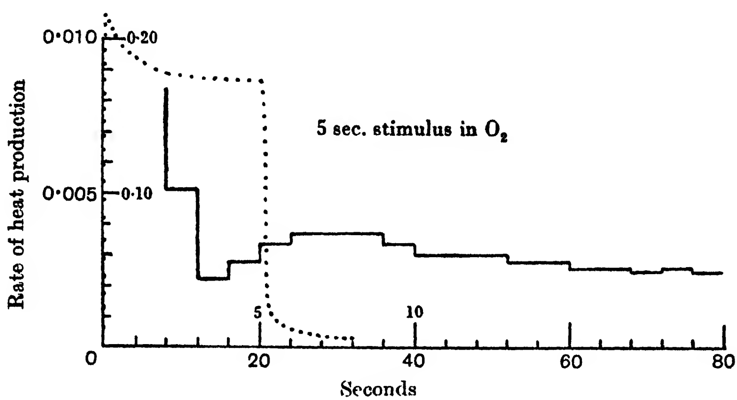

Fig. 1. Rate of heat production for a 5 sec. stimulus in oxygen at 17° C. The dotted line, with inner scale, gives the result of the initial analysis up to 8 sec., showing the characteristic high rate soon after a long stimulus. The tension fell only slightly during the stimulus; at 0·4 sec. after the stimulus it had fallen to a very small value, at 0·6 sec. it was inappreciable.

The full line, with outer scale, gives the result of the subsequent analysis in 4 sec. steps up to 80 sec.; this is the continuation of the dotted curve on 20 times the scale, showing a distinct maximum at about 30 sec. due to oxygen recovery, whereas the earlier quickly falling part is almost entirely due to anaerobic delayed heat.

The unit for the vertical scale is the initial heat per sec.

Such a result has been observed before [Hartree and Hill, 1928, Fig. 3, p. 213], but its significance was not then understood. It is now found that if a similar long stimulus be given in nitrogen (freed from oxygen by passing over heated copper) the part of the delayed heat curve soon after relaxation is practically unaltered, and there is no subsequent rise, in fact there is no observable heat at all after 15–20 sec. It is clear, therefore, that the heat soon after relaxation is nearly entirely due to the same cause as the anaerobic delayed heat, so no proper investigation of the heat in oxidative recovery can be made without first deter-

mining the anaerobic delayed heat for various times of stimulus and deducting this from the total heat observed after relaxation.

This early delayed heat represents presumably the excess of the heat due to delayed lactic acid formation over that absorbed in delayed phosphagen resynthesis [Lehnartz, 1931; Lundsgaard, 1931; Meyerhof and Schulz, 1931], and this theory of its origin is borne out by the fact that Meyerhof and Schulz found that the lactic acid formed during and immediately after the tetanus, as also the phosphagen resynthesized during the same period, is the same in a muscle saturated with oxygen as in one which is entirely oxygen-free. We are justified, therefore, in assuming the same source for the early delayed heat in both cases.

The anaerobic delayed heat. There was some trouble in making a sufficiently good determination of the anaerobic delayed heat because it was soon evident that it was less, and over earlier, for successive stimuli, and with long stimuli generally much less for the later ones as has been noted elsewhere [Cattell and Hartree, 1932]. For example (16. x. 31) several stimuli of 1 sec. gave

No. 1. A.D.H. = 11 p.c. over in 40 sec.
No. 3. A.D.H. = 4 p.c. over in 20 sec.
No. 8. A.D.H. = 2 p.c. over in 4 sec.

Also (16. ix. 31) three 4 sec. stimuli, after others, gave

No. 3. A.D.H. = 9 p.c. over in 20 sec.
No. 4. A.D.H. = 7 p.c. over in 16 sec.
No. 5. A.D.H. = 3 p.c. over in 12 sec.

Thus it was necessary to have an early stimulus in several different experiments to obtain consistent results. Fig. 2 shows the mean results for an early stimulus from twelve experiments. In all these the total anaerobic delayed heat (after completing the diagram for the shorter stimuli)[1] was about 8 p.c. of the initial heat. No greater anaerobic delayed heat than 12 p.c. was obtained.

There is some evidence that about 2 p.c. of the initial heat occurring in a few seconds after the tension has fallen to its original value may be due to the relaxation from internal stresses which do not contribute directly to the tension, as such heat is frequently observed after shorter stimuli when both the anaerobic delayed heat and the oxygen recovery heat are probably at a very small rate and cannot account for it; and further, this 2 p.c. occurs at a falling rate before the subsequent rising rate associated with the anaerobic delayed heat or the recovery heat.

[1] For stimulus 0·8 sec. the anaerobic delayed heat seems to be practically complete in about 50 sec. and for stimulus 0·4 sec. in about 80 sec.

After a long stimulus, the tension when the stimulus is over does not usually fall quickly to its original value but may be quite appreciable for a few seconds: such tension is small and the heat due to relaxation from it is not likely to reach 1 p.c. (it is probably considerably less): the above considerations, however, suggest that the anaerobic delayed heat has, if anything, been over-estimated and that after comparatively few long stimuli its total amount is inappreciable. The possibility, however, must not be overlooked that for the subsequent stimuli the anaerobic delayed heat may have become so early in its appearance that it cannot be separated from the initial heat.

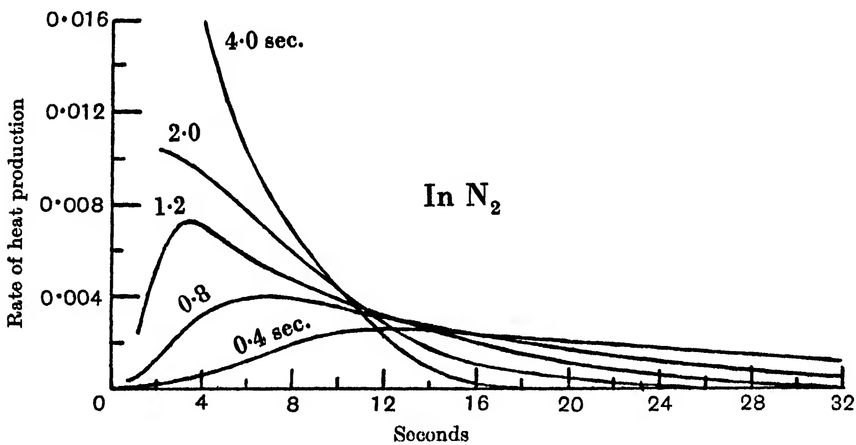

Fig. 2. Curves showing the mean relative heat rate for early stimuli of 0·4, 0·8, 1·2, 2·0 and 4·0 sec. at 17° C. in N_2, from the results of twelve experiments. The curve for 0·8 sec. stimulus goes on for about 50 sec., that for 0·4 sec. stimulus for about 80 sec. The total heat (area) for each curve is about 8 p.c., except for the 4 sec. stimulus in which it is less, as a considerable amount of the heat came before the end of the stimulus. The curves, especially before 4 sec., are approximate; the results of any one experiment sometimes differed considerably from them. The unit for the vertical scale is the initial heat per sec. for each curve.

For longer stimuli the initial rate is very high, which fully accounts for the distinctive "tail pieces" in all the heat diagrams after such stimuli. For a 4 sec. stimulus the maximum rate probably occurs before the end of the stimulus though it cannot be separately observed, but even after this the rate is actually five times the maximum possible rate due to oxygen recovery (see later), although the whole anaerobic delayed heat is only about 8 p.c. of the whole oxygen-recovery heat. After a stimulus of this length the anaerobic delayed heat is complete in under 20 sec., which agrees with the fact that many observers have found the phosphagen resynthesis and the delayed lactic acid formation to be complete in a comparatively short time.

Fig. 2 shows the relative rates of anaerobic delayed heat, *i.e.* the ordinates of the curve for each stimulus are fractions of the initial heat for that stimulus. If these rates be required in absolute units they must be multiplied in each case by the initial heat.

A calibration was made in several of the experiments for the anaerobic delayed heat, and also in several for the oxygen-recovery heat (considered later); the mean values of the initial heat for various times of stimulus, from a single twitch up to 4 sec., were plotted against the time of stimulus: from the curve the initial heats for the times of stimulus used in Fig. 2 were read off and these are entered in Table I, together with the maximum rate of anaerobic delayed heat.

TABLE I. Maximum rates of anaerobic delayed heat for different initial heats.

Time of stimulus (sec.)	0·4	0·8	1·2	2·0	4·0
Initial heat (10^{-2} cal. per g.)	3·3	5·0	6·9	11·1	19
Maximum relative rate	0·0025	0·004	0·007	0·010	0·020*
Maximum absolute rate (10^{-4} cal. per g. per sec.)	0·8	2·0	4·8	11	38
Approximate time to maximum rate (sec.) ...	14	7	3	2	—

* The figure 0·020 is an estimate, since the maximum rate for a 4 sec. stimulus probably occurred before 4 sec.

From the numbers it may be seen that the maximum absolute rate of anaerobic delayed heat is closely proportional to the square of the initial heat preceding it.

The oxygen-recovery heat. Having determined the curves of anaerobic delayed heat rate for early stimuli of various durations these were subtracted from the curves for corresponding stimuli when the muscle was in oxygen in order to get the oxygen-recovery heat alone. It seems reasonable to subtract such anaerobic delayed heat curves from the mean curves for various times of stimulus using all the observations in oxygen, on the supposition that when the muscle was allowed to recover the anaerobic delayed heat effect would always be the same as for an early stimulus in nitrogen, which corresponds to the case of the fresh muscle.

The mean curves for the relative rates of oxygen-recovery heat are shown in Fig. 3 and the absolute rates in Fig. 4. For even the longest stimulus used, namely 4 sec., the maximum rate does not occur before 20 sec. The relative rate is considerably less for the longest stimulus, and it seems that in this case the absolute rate is approaching its maximum possible value (see Fig. 5).

Shorter stimuli were also given in oxygen with a view to finding the recovery rate for a single twitch. In this case the maximum relative

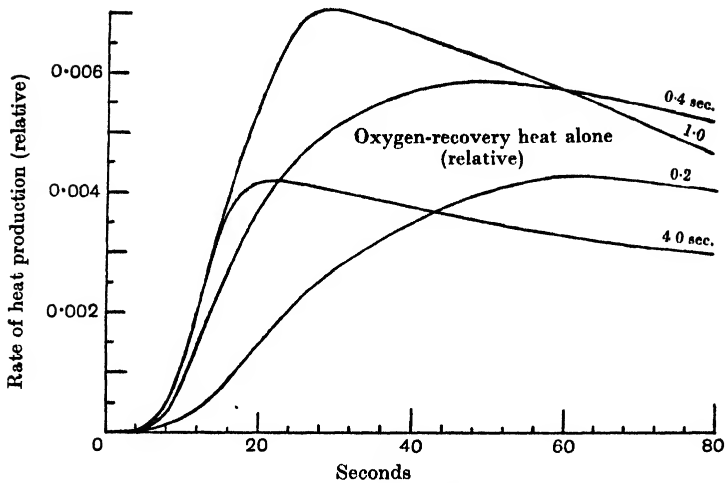

Fig. 3. Curves showing the mean relative rates of oxygen-recovery heat for stimuli 0·2, 0·4, 1·0 and 4·0 sec. at 17° C., from the results of sixteen experiments. The means of three or four analyses for each time of stimulus (in several different experiments) were taken and the anaerobic heat, deduced from Fig. 2, subtracted in each case. The unit for the vertical scale is the initial heat per sec. for each case.

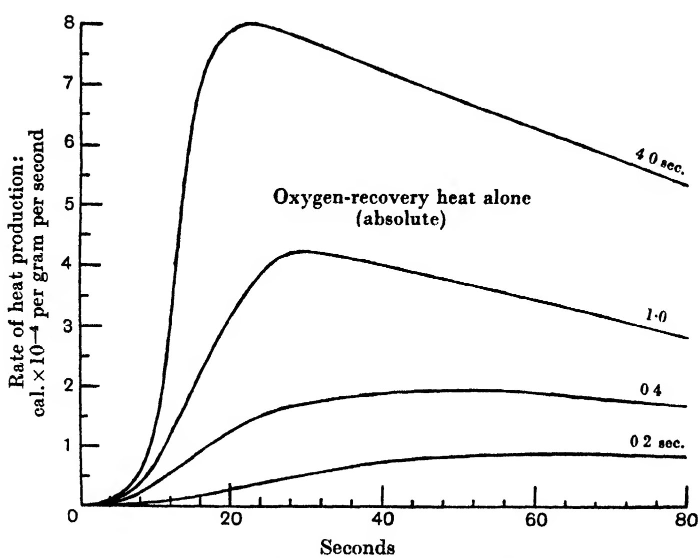

Fig. 4. Curves showing the mean absolute rates of oxygen-recovery heat for stimuli 0·2, 0·4, 1·0 and 4·0 sec. at 17° C. These are the same curves as in Fig. 3, but each multiplied by the corresponding initial heat.

rate of total recovery (including the anaerobic delayed heat) was about 0·002 of the initial heat per sec., giving an absolute maximum rate of about 10^{-5} cal. per g. per sec., and this occurred at about 80 sec. In this case of course the galvanometer had to be made much more sensitive

TABLE II. Maximum rates of oxygen-recovery heat for different initial heats.

Time of stimulation (sec.)	0·2	0·4	1·0	2·4	4·0
Initial heat (10^{-2} cal. per g.)	2·1	3·3	5·9	12	19
Maximum relative rate	0·0043*	0·0058	0·0070	0·0062	0·0042
Maximum absolute rate (10^{-4} cal. per g. per sec.)	0·9	1·9	4·1	7·4	8·0
Approximate time to maximum (sec.)	65	50	30	—	22

* The anaerobic delayed heat was not observed for stimuli less than 0·4 sec., so this number which required an extrapolation is doubtful, as are the further numbers in the same column.

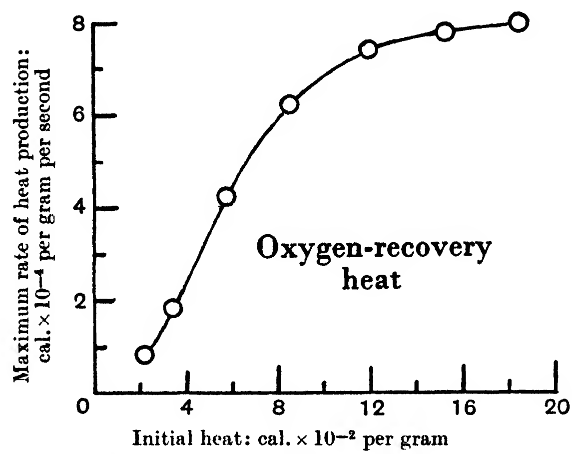

Fig. 5. Showing the maximum absolute rates of oxygen-recovery heat at 17° C., after different initial heats for times of stimuli 0·2, 0·4, 1·0, 1·6, 2·4, 3·2 and 4·0 sec. The maximum possible rate of oxygen-recovery heat at 17° C. cannot apparently be much greater than 8×10^{-4} cal. per g. per sec. and this is practically reached after a stimulus of 4 sec. The plotted points are the means from about forty results in sixteen different experiments.

than when dealing with the longer stimuli, so the small differences (between the live and the control curve at some time after the stimulus) on which the method depends, were not so reliable: it was therefore considered impossible to make reliable determinations of the anaerobic delayed heat in such a case, and it can only be stated that after a single shock the maximum oxygen-recovery heat rate is somewhat less than 10^{-5} cal. per g. per sec. and that this does not occur before $1\frac{1}{2}$, or possibly

2 min. after the stimulus. This time is longer than that given above, as the effect of deducting the anaerobic delayed heat from the total recovery is to make the maximum of the resulting curve (the oxygen-recovery) come at a later time than that of the original curve.

The maximum rate of oxygen-recovery heat production shown in Fig. 5 approaches asymptotically a value of about 8×10^{-4} cal. per g. per sec., for the temperature of the present experiments (17° to 17½° C.). There is a limit, it seems, in the rate of oxidative heat production, set presumably by the maximum possible activity of the enzymes or other factors concerned in oxidation. The limit is not set by the amount of oxygen available: a fully recovered muscle at 20° C. in oxygen should contain [Hill, 1928 *b*, p. 47] about 90 p.c. of the full amount its water would dissolve at 1 atmosphere partial pressure, say, about 0·022 c.c. per g. This would allow about 0·1 cal. of energy to be liberated per g., taking no account of further oxygen diffusing in: of the 0·1 cal. about one-half (0·05 cal.) presumably would appear as heat, the other half would be absorbed in the endothermic processes of recovery. According to Fig. 4 (4 sec. curve) not more than about one-fifth of this 0·05 cal. is liberated up to the time that the maximum is obtained, so that presumably nearly all the oxygen is still available at that time.

Discussion.

All the experiments were carried out on a pair of sartorii of *R. temp.*, and in most cases the muscles were small or very small: for a constant length of 21½ mm. between the stimulating electrodes, the weight of the pair of muscles was always between 30 and 70 mg. (except for one or two cases in which a single twitch was used when they were somewhat larger). The average thickness of each muscle was 0·4 mm. estimated from the weight, length and mean breadth. The small size of the muscles ensured rapid distribution of heat and rapid diffusion of oxygen from outside. The temperature was always between 17° and 17½° C.

In nearly all the experiments on anaerobic delayed heat there was an apparent small heat production at the end of a considerable time; in fact there was frequently a very small rise in the galvanometer deflection after about 60 sec. There was of course always the possibility that such a result might be due to a temperature error in the course of the record, but on several occasions the spot was observed for a considerable time after the muscle was dead or had not been stimulated for a long interval, and there was no observable motion. The apparent

small heat production referred to is no doubt a vapour-pressure effect that has been observed in other cases [Hill and Kupalov, 1930], so this final part of the calculated heat has never been included in the results, but the heat rate has been smoothed off at the end on the supposition that such final parts are due to this technical error. The times, therefore, referred to above in which the anaerobic delayed heat is stated to be complete, must be accepted with some reserve since they depend on an estimate of the final error.

The form of the curves in Figs. 3 and 4 for the true oxygen-recovery heat rate makes it clear that the recovery process does not immediately start off at its maximum value but passes through a complicated curve, of the meaning of which we have at present little idea. When more is known about the chemical events occurring in recovery they will presumably fit into the outline shown in these figures. That the anaerobic delayed heat occurs so rapidly after the longer stimuli and so relatively slowly after the shorter ones also has no explanation at present, but it obviously has an important bearing upon observations made by chemical methods on the processes occurring after activity. It is satisfactory (*a*) that the duration of the anaerobic delayed heat appears to be about the same as that of the time during which lactic acid formation occurs and phosphagen restoration is effected, and (*b*) that the heat which we have called the anaerobic delayed heat occurs also after stimulation in oxygen and so conforms to the chemical facts now known about lactic acid and phosphagen. That it was present in both cases had in fact been seen before [Hartree and Hill, 1928] and had been assumed whenever anaerobic delayed heat was subtracted from oxygen recovery heat in finding the ratio of recovery heat to initial heat.

The ratio (heat in oxygen) : (heat in nitrogen) for a series of muscle twitches [Hill, 1928 *a*] is about 2·07. If H_i be the initial heat, H_o the oxygen-recovery heat, H_a the anaerobic delayed heat, then the heat in oxygen is $(H_i + H_o + H_a)$ and the heat in nitrogen $(H_i + H_a)$. Hence $H_o/(H_i + H_a) = 1{\cdot}07$. In previous investigations [Hartree and Hill, 1922, 1924; Hartree and Liljestrand, 1926] the ratio of total delayed heat to initial heat $(H_o + H_a)/H_i$ has been determined by methods less exact indeed than those adopted in the present investigation but still probably good enough for total heats. Now if the results of the present experiments on tetani can be applied to series of twitches, $H_a = 0{\cdot}08\, H_i$. Hence $(H_o + H_a)/H_i = 0{\cdot}08 + H_o/H_i = 0{\cdot}08 + 1{\cdot}08 \times 1{\cdot}07 = 1{\cdot}24$. This is not far from the average of previous estimations.

An apparent negative delayed heat.

Since the anaerobic delayed heat at 17° C. after a long stimulus is of such high initial rate it seemed possible that determinations of it might be made at 0° C. In five experiments on English and four on Dutch frogs at 0° C. in nitrogen, stimuli of 1–5 sec. were given. In every case with 1 sec. stimulus the analysis, carried out as explained above, gave apparent negative heat after relaxation; the average amount was -3 p.c., lasting for about 40 sec., and there was a distinct tendency for this negative heat to become numerically greater after successive stimuli, the variation being usually from about -1 p.c. for fresh muscle to about -5 p.c. after a few stimuli of 1 sec., and to a somewhat greater negative number when a 1 sec. stimulus was given after a longer stimulus (4 or 5 sec.).

If real, this delayed negative heat in the absence of oxygen would be very interesting: it might be a sign of the endothermic restoration of phosphagen outstripping the exothermic one of lactic acid formation: there is no *a priori* reason why at some stage in this double process the total energy (as distinct from the free energy) should not be negative. The negative heat, however, was very small and it was necessary to see whether it could be explained as a technical error of some kind.

If the outer layers of the muscles (*i.e.* those further from the face of the thermopile) were less active than the rest, their rise of temperature, after a stimulus, would be less than that of the rest, so they would take heat from the inner part and this would appear in the analysis as delayed negative heat. The redistribution of heat in such a case of non-uniform contraction was investigated mathematically by Hill [1931, Appendix II]: it appeared that the process was so rapid that it would not affect the analysis after a very few seconds, and so could not be the cause of the effect observed.

It was safer, however, to examine the matter experimentally as well, which was done as follows. Each muscle of a pair was covered with a layer of absorbent paper to provide an "inactive layer" which would be heated when the controls were taken; the muscles were then given a short stimulus (0·2 sec. at 17° C.) and the heat was analysed in the usual way.

When thick blotting paper was used weighing (wet) 1·4 times as much as the muscles, the apparent negative heat was about -60 p.c. of the initial heat, and it was inappreciable within 3 sec. after relaxation; when thin cigarette paper was used weighing (wet) 0·15 × (weight of muscles), the apparent negative heat was about -18 p.c. of the initial heat and it was inappreciable within 1½ sec. after relaxation. The equalization of temperature was so rapid that the experiments had to be made at a high temperature, and even then some of the negative heat overlapped the relaxation heat; an approximate allowance for this was made when deducing the above numbers.

Since the apparent negative heat was at its greatest rate within a few tenths of a second after the stimulus, and inappreciable after the short times given above, it was clear that inactivity of the outer layers could not account for the long-continued negative heat observed at 0° C.

A more probable explanation is as follows. Suppose that, when the control curve was taken, the muscles (owing to shortening or swelling in killing[1]) were of greater weight than when the live curve was taken. The heavier muscles would lose temperature less

[1] The muscles were always killed with chloroform vapour, but this was always so weak that, apart from one or two exceptional cases, there was no sign of contracture and no change in the appearance of the muscle. The chloroform vapour was usually removed from the thermopile chamber before the control curves were taken.

rapidly than the lighter ones, so the control curve taken on the former would be too high at all times after the maximum and, as the analysis of the initial heat would be hardly affected, the calculated allowances for the effect at all subsequent times of the initial heat would be too great, so that the result of subtracting them from the live curve would be negative.

To test this, curves were taken consequent on heating (for 0·1 sec.) a pair of strips of thick blotting paper (wetted with Ringer's solution) and also for the same blotting paper covered with a layer of thinner absorbent paper. When the latter was filter paper, giving an increase of weight of 39 p.c., the apparent negative heat, in an analysis of the first curve by the second, was about -25 p.c. of the initial heat and lasted about 110 sec.; when the thinner paper was cigarette paper, giving an increase of weight of $12\frac{1}{2}$ p.c., the corresponding negative heat was about -8 p.c. of the initial heat, and lasted for about 70 sec.

In each case the negative rate fell rapidly from an early high value so that about one-third of the whole negative heat occurred in the first 4 sec. Except for this, the distribution was somewhat similar to that of the negative heat observed at 0° C., but a close comparison cannot well be made, since at 0° C. the end of the relaxation heat may overlap the beginning of the negative heat and the usual anaerobic delayed heat may overlap the end of it. It must be remarked, however, that in many cases after a 1 sec. stimulus at 0° C. there was a very perfect "wash out" at the end of the initial analysis, so that there was no observable heat of any kind between 3 and 4 sec.; in this interval the relaxation heat can hardly have had an appreciable effect (unless, as has been suggested elsewhere, there is a small amount of relaxation heat after the tension has fallen to its original value) and the negative heat, if due to a difference of weight as discussed above, would then be at about its greatest rate, so it could hardly escape detection.

Another possible objection to this explanation of the negative heat lies in the fact that the negative heat for stimuli at 0° C., invariably increased (numerically) for successive stimuli of the same duration, so that it would be necessary to assume that the usual anaerobic delayed heat masked it less and less for successive contractions: but it has been noted above that at 17° C. the anaerobic delayed heat does diminish in this way.

In several cases of longer stimulus (4 or 5 sec.) at 0° C. the negative heat, though present in every case, was always less (when reckoned as a fraction of the initial heat) than for a short stimulus: this may have been due to the earlier occurrence of the anaerobic delayed heat, which might be expected after a longer stimulus.

In five experiments at 0° C. the muscle was poisoned with iodoacetic acid (1 hour at room temperature and $\frac{3}{4}$ hour at 0° C. in 1/25,000 iodoacetic acid, which gave a good contracture after repeated stimulation in a muscle separately tested). For an e a r l y stimulus of 1 sec. at 0° C., the apparent negative heat was very uniform, -2 to -3 p.c.; for later stimuli there was a conspicuous progressive change due to subsequent positive heat occurring earlier and at a higher rate. The fact that iodoacetic acid neither abolishes nor increases the negative heat, suggests that this heat is not due to a balance between phosphagen restoration and lactic acid formation, for in the poisoned muscle the latter does not occur.

On the balance of evidence it seems most likely that the negative heat is due to a small increase of weight between "live" and "control." It is hoped to test this by a method (used by Dr E. Bozler for the snail's retractor) employing a high-frequency current which will allow "controls" to be taken, mixed with "live" records, on the living muscle. If the negative delayed heat prove to be an error due to change in weight of muscle, it must be remarked that all published determinations (including the present) of the anaerobic delayed heat are probably slightly too small. The oxygen-recovery curves, however, would be unaffected, since they were obtained by subtraction.

SUMMARY.

1. A detailed analysis has been made of the delayed heat production occurring after a muscular contraction.

2. In a fresh muscle deprived of oxygen this delayed heat amounts to about 8 p.c. of the initial heat; it occurs earlier and at a greater rate, and ends sooner, after longer stimuli. After a 4 sec. tetanus at 17° C. it is complete in about 20 sec. In successive stimuli the amount decreases, or perhaps occurs earlier so that it cannot be so completely separated from the initial heat.

3. In a muscle in oxygen the delayed heat occurs in two phases: (*A*) the anaerobic delayed heat, which appears exactly as in a muscle without oxygen: (*B*) the true oxygen-recovery heat. In the present experiments (*B*) has been separately examined by subtracting (*A*) from the total in oxygen.

4. The true oxygen-recovery heat rate rises to a maximum and then slowly declines. The maximum is earlier and higher the greater the initial heat. The greatest rate of oxygen-recovery heat production approaches the value (at 17° C.) 8×10^{-4} cal. per g. of muscle per sec. This maximum is determined by a limit, not in the rate of supply of oxygen, but in the rate of chemical reaction.

5. The anaerobic delayed heat represents a balance between the endothermic resynthesis of phosphagen and the exothermic formation of lactic acid. These processes occur equally in the presence or the absence of oxygen, and are complete in a time about equal to that of the heat after the longer stimuli.

6. The oxygen-recovery heat represents a balance between exothermic oxidation and endothermic lactic acid removal and phosphagen resynthesis.

7. The observation of an apparent negative delayed heat is discussed.

I am much indebted to Mr T. P. Feng for his valuable assistance in the laborious task of working out many of the earlier experiments, and to Prof. A. V. Hill for his advice and for his help in the preparation of this paper.

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PULMONARY ŒDEMA IN THE CAT HEART-LUNG PREPARATION.

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RESULTS OF PREVIOUS OBSERVERS.

On the intact animal. The experimental methods employed to produce lung œdema in animals have been diverse, and the results obtained various. A résumé of the literature up to 1915 was given by Matsuoka [1915]. It is sufficient to say with regard to this, that the possibility of producing œdema by obstructing the outflow from the left side of the heart, or rendering it incompetent in various ways, was fairly well established, but observers differed as to the importance to be attached to the condition of the lung capillaries.

On the heart-lung preparation. Matsuoka [1915] found that œdema could be produced in the dog heart-lung preparation in 15 minutes by making the heart put out 1½ litres per minute against a low arterial resistance of 80 mm. Hg. The same result was obtained by making the heart work for 15 minutes against a resistance of 160 mm. Hg, with a low output of 630 c.c. per minute. He designates this type of œdema as "obstructive," the common denominator in the two cases being a failure of the left ventricle, not shared by the right, to deal with the blood coming to it, resulting in a distension of the lung capillaries. Barry [1923], from similar experiments, concluded that the principal factor was a low colloid osmotic pressure of the blood, and that the effects of flow and resistance were merely contributory. His evidence, however, was mainly from experiments in which the colloid osmotic pressure had been deliberately lowered by adding saline. Lambert and Gremels [1926], also working on the dog heart-lung preparation, attribute the œdema to a toxic action of defibrinated blood upon the capillaries. Neglecting their method of recording the onset of œdema, which is open to criticism, the main conclusion of these authors is well supported by their experiments.

On the isolated lungs. Using cat lungs, Modrakovski [1914] showed that, before œdema could be produced, the perfusion pressure had to be raised above 35 mm. Hg, and the venous outflow obstructed so that the fall of pressure through the lungs was only 8 mm. Hg. Matsuoka [1915] demonstrated similar relationships in dog lungs, giving 40 mm. Hg as the critical perfusion pressure; he regards the rise of pulmonary arterial pressure in the heart-lung preparation as the result of a damming back of blood by the left side of the heart, and presumably assumes that when the critical pressure is exceeded, œdema occurs.

Preliminary considerations.

There is no reason to assume any essential difference between the pulmonary œdema of the dog heart-lung preparation and that of the cat heart-lung preparation. The latter was chosen as a basis for the present experiments because of its very early and almost inevitable appearance [Evans, 1912; Knowlton and Starling, 1912 *b*; Gilding and Newton, 1931[1]].

As a criterion of œdema the appearance of fluid in the tracheal cannula has been taken. This necessarily indicates an advanced stage, but there seems to be no satisfactory measurement applicable to the finer degrees. Lambert and Gremels [1926] claimed that the electrical resistance of the lungs was independent of the blood flow, and varied inversely as the degree of œdema. It is difficult to see how a record of this can discriminate between an accumulation of fluid in the alveoli and an accumulation of blood in the capillaries, and as congestion is a possible causative factor, the method has not been tried. Hæmatocrit determinations on the circulating blood [Smirk's method, 1928] showed a progressive concentration of corpuscles, but as a test for the onset of œdema this was of no more value than the actual appearance of fluid.

The visible changes in the lungs are probably significant. Immediately after the heart-lung circuit is established, they distend less at each thrust of the pump, and their pink colour changes to a much deeper shade. There is then a progressive increase in volume at each thrust, the pleural surface exudes serum, blood is lost from the reservoir, and the circulating blood becomes more venous in colour. The lungs collapse very badly, and finally blood-stained fluid appears in the tracheal cannula.

[1] Unpublished observations.

FIRST SERIES OF EXPERIMENTS.

Effect of positive ventilation. There was no sign of œdema in eight cats anæthetized with chloralose (0·1 g. per kg. of body weight intraperitoneally), and subjected to positive ventilation with open thorax for over 2 hours. The latter was combined variously with ligature of the brachiocephalic artery, vagal section, decapitation, suprarenalectomy, defibrination of the blood, and addition of anti-coagulant to the blood (B.D.H. chlorazol sky blue, 8 p.c. solution, 2 c.c. per kg. of body weight intravenously). The uniformly negative results suggested that the cause of œdema was to be sought in some factor introduced by the actual heart-lung apparatus.

Several heart-lung preparations were ventilated with 5 p.c. CO_2 in air or oxygen, to prevent acapnia, but the incidence of œdema remained unaffected.

Trauma of blood. In one cat, pulmonary œdema was obtained by repeatedly removing, whipping and re-injecting the blood, even after fibrin ceased to be deposited. The significance of this experiment is doubtful, as about 75 c.c. of blood from another cat were added to keep up the blood-pressure, and the result could not be repeated on two occasions when the amounts of blood removed and re-injected were exactly the same. Also, in several of this series, and all of the next series of experiments, whipping was rendered unnecessary in preparing the heart-lung by the use of Chicago blue (chlorazol sky blue), but œdema occurred with the same frequency.

Influence of foreign blood. No agglutination was ever observed between the blood of the heart-lung cat and that of the cat used for bleeding. Attempts to make a heart-lung preparation from a single cat were not satisfactory, owing to the small amount of blood available.

Other possible causes. Irritation due to the use of volatile anæsthetics was ruled out by the use of intraperitoneal chloralose in all experiments.

Free lymphatic drainage was assured in a number of heart-lung preparations by severing the superior vena cava distal to the inflow cannula; the incidence of œdema was not affected.

Influence of venous inflow and resistance. In all of seven preparations, with an arterial resistance of 150 mm. Hg and a maximal venous inflow (about 320 c.c. per minute), œdema supervened rapidly, the average time being 30 minutes from the completing of the heart-lung circuit. Of sixteen preparations, with an average arterial resistance of 85 mm. Hg and a venous inflow of 100 or less c.c. per minute, only eight developed

œdema within 1 hour, and in most of the others the preparation was in good condition at the end of 2–2½ hours. These results confirmed those of other workers [Matsuoka, 1915; Knowlton and Starling, 1912 *a*; Barry, 1923], but no attempt was made to find the "critical" inflow or resistance, for the experiments did not suggest that these were of primary importance. The normal blood-pressure of a cat is considerably more than 85 mm. Hg, and its heart must ordinarily be able to deal with much more than 100 c.c. of blood per minute. Yet in one half of the experiments, where these values obtained, œdema came on rapidly, and in some of the remainder appeared ultimately.

Second series of experiments.

The last group of results leaves no doubt that the conversion from the normal to the heart-lung circulation predisposes the preparation in some way to œdema, and that this occurs earlier if an increased load is placed upon the heart. With the object of finding whether the conversion is attended by some abnormal distribution of pressures, these were followed through from the normal to the heart-lung circulation: aortic pressure (subclavian artery, Hg manometer), left auricular pressure (left auricular appendix, saline manometer, tank recorder), pulmonary arterial pressure (upper branch of right pulmonary artery, Hg manometer), superior vena cava pressure (azygos vein, saline manometer, tank recorder).

Procedure. After the anæsthetic (0·1 g. choralose per kg. body weight, intraperitoneally) the main steps were as follows: tracheal cannula inserted, thorax opened and artificial respiration turned on; heart-lung ligatures placed in position; cannulæ placed in left subclavian artery, azygos vein, and upper branch of right pulmonary artery; Chicago blue injected into tibial vein, and blood released into the pressure cannulæ; pericardium snipped, and cannula rapidly inserted into left auricular appendix; tracing of normal pressures taken; cannulæ inserted into brachio-cephalic artery and superior vena cava; aorta (beyond subclavian) and inferior vena cava tied and the heart-lung circuit thrown in.

Apparatus. The general plan of this is too familiar to require description, comprising as usual an air cushion, arterial resistance, warming spiral, and venous reservoir. It was adapted to the present experiments by three modifications: (*a*) The whole was immersed in a water bath, filled to a constant level, and kept at 38–40° C. (*b*) The venous reservoir was fitted with a "keel" of lead piping, and floated in the bath; this ensured that the contained blood, whatever its amount, was always at the same level above the table (very near approximation). (*c*) Between the reservoir and the venous cannula, a glass tap, with a long arm and circular scale, was inserted. This enabled the inflow into the preparation to be controlled, and as the blood passing through the tap was at a constant head of pressure, the actual rate of inflow was known from the position of the tap.

For these experiments, the venous inflow tap was kept in a position corresponding to a flow of 115 c.c. per minute. This rate, however, is that

observed with the venous cannula at table level, and emptying against zero pressure. The actual rate of inflow at any moment during an experiment depends upon the height of the superior vena cava above the table, and the pressure in this vessel as recorded from the azygos vein. The corrected inflow was read off from a chart, on which the flow was plotted against the height of the cannula above the table; it was assumed that a pressure of x mm. of saline in the superior vena cava, itself y mm. above

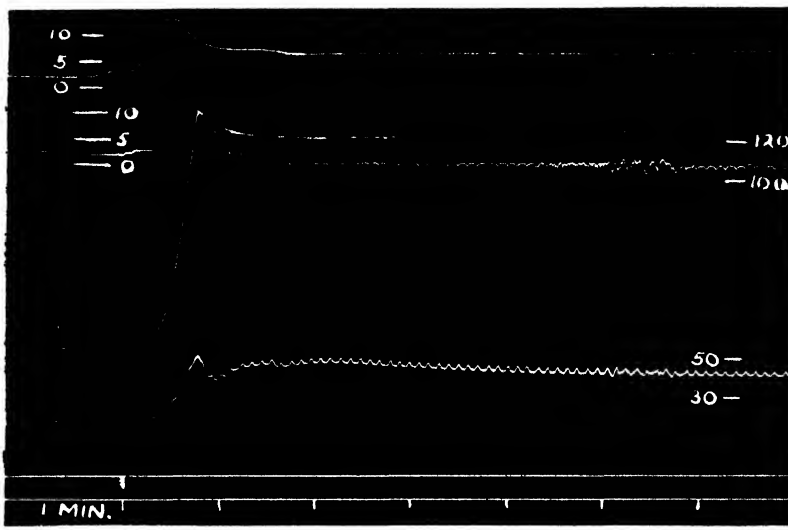

Fig. 1. Showing changes of pressure on establishment of heart-lung circuit; on the right is seen evidence of temporary arrhythmia followed by a fall in aortic and a rise in left auricular pressure. From above downwards: superior vena cava, left auricular, aortic and pulmonary arterial pressures. Left-hand scales in cm. H_2O. Right-hand scales in mm. Hg.

the table, was equivalent to a height of $x + y$ mm. of the open cannula above the table.

The arterial resistance was always in the neighbourhood of 100 mm. Hg.

Results. Fig. 1 shows that the change-over itself is associated with no pressure changes but what might be expected, with the exception that the pulmonary arterial pressure is rather high. The abnormal level of this (30–40 mm. Hg) was a constant feature; it was higher in the heart-lung preparation where the resistance was 100 mm. Hg than when the circulation was intact and the systemic pressure twice this figure, *e.g.* just after tying off the brachio-cephalic artery.

Shortly after the heart-lung circuit was established, the tracings and the appearance of the heart indicated a failure of the cardiac muscle. The heart became dilated, the output and aortic pressure fell, and the left auricular and superior vena cava pressures rose. The pulmonary arterial pressure rose, or was already high. Usually the left side of the heart

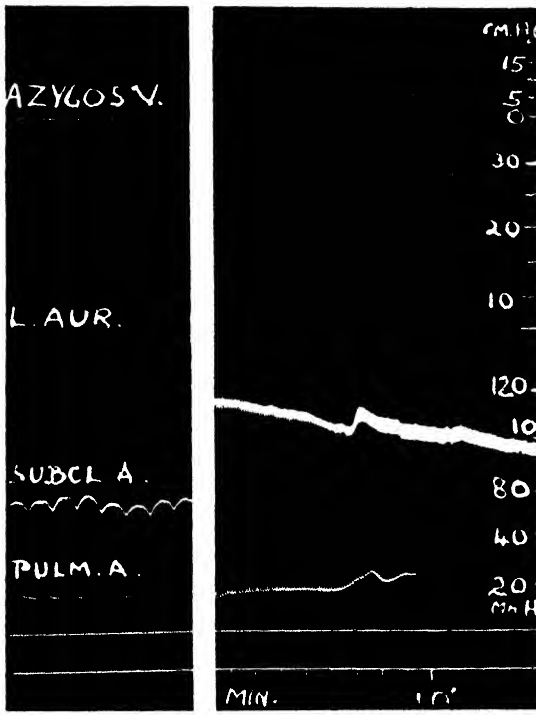

Fig. 2. Left-hand tracing: pressures before establishment of heart-lung. Right-hand tracing: pressures immediately after establishment of heart-lung, showing sudden rise in left auricular pressure, with a falling aortic pressure. The secondary variations are due to attempts to restore normal pressures by addition of glucose and insulin. Upper scales in cm. H_2O; lower in mm. Hg.

failed before the right, as is shown in Fig. 2, where the rise in left auricular and fall in aortic pressure take place before there is any appreciable change in the vena cava. Sometimes the reverse was the case (Fig. 3), and frequently the left auricular and vena cava pressures went up together. In a number of experiments these events were temporary, and the heart settled down to a steady condition with both venous pressures somewhat raised.

The effects of adding adrenaline and glucose (isotonic) to the venous reservoir are shown in Figs. 4 and 5 respectively. In both cases the venous

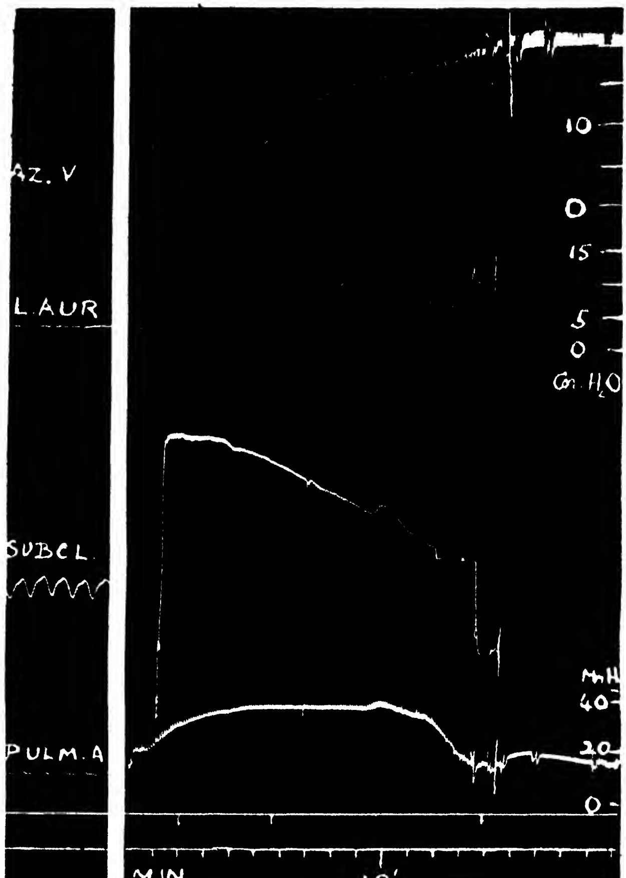

Fig. 3. Left- and right-hand tracings: pressures immediately before and immediately after establishment of heart lung, respectively. Right-sided heart failure shown by rising pressure in superior vena cava. The final drop in pulmonary arterial pressure, and the transmission of ventricular pulsations to the vena cava, indicate complete valvular incompetence. The falling aortic pressure is chiefly due to diminished inflow from right heart. Upper scales, cm. H_2O; lower scale, mm. Hg. Signals, left to right; outputs, 95 c.c. per min., 60 c.c. per min., and 0 c.c. per min. Last signal also gives alignment of tracings.

pressures were lowered, and the output and aortic pressure raised. Glucose had no marked effect upon the pulmonary arterial pressure, while adrenaline either raised or lowered this.

The addition to the reservoir of blood, which had been kept for a day or more in the ice chest, was observed to produce in an exaggerated degree the same effects which have been described as taking place shortly after the establishment of the heart-lung preparation. Fig. 6 shows the termination of the experiment in which this was first noticed. Careful examination of tracings revealed a suspicion of the same results when blood,

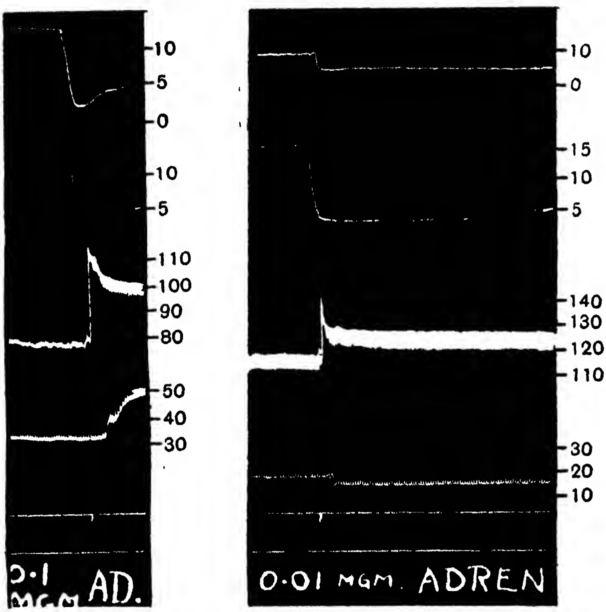

Fig. 4.

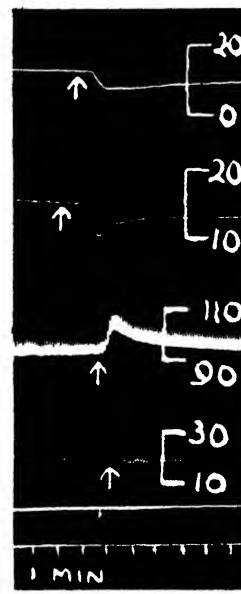

Fig. 5.

Fig. 4. Tracings from above downwards in both experiments: superior vena cava, left auricular, aortic and pulmonary arterial pressures. Upper scales in cm. H_2O, lower scales in mm. Hg. The effect of adrenaline.

Fig. 5. Order of tracings as in Fig. 4. Effect of adding 25 c.c. isotonic glucose solution to reservoir.

which had been kept in reserve only from the beginning of the experiment, was added to the reservoir. The procedure hitherto observed in making the preparation was accordingly modified, in that the blood used for the initial filling of the reservoir was taken from the second cat after all the cannulæ were in position, and not as usual before the heart-lung operation was commenced. After the intravenous injection of Chicago blue, the cat was bled into a beaker and the blood poured through a gauze filter, straight into the venous reservoir; only a few minutes elapsed

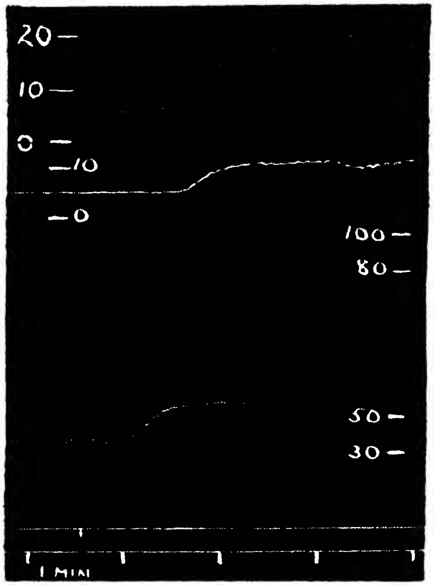

Fig. 6. Order of tracings as before. Left-hand scales in cm. H_2O, right-hand scales in mm. Hg. 30 c.c. blood having been removed from the reservoir, 30 c.c. 24-hour-old blood (from ice chest) warmed and added to reservoir. Note rise in pulmonary arterial pressure and failure of both sides of heart.

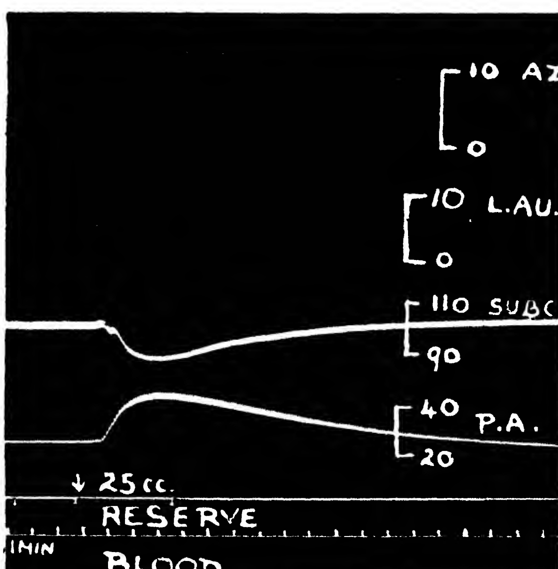

Fig. 7. Effect of adding to the reservoir 25 c.c. blood, kept for one hour, and taken originally from the same cat at the same time as the blood already in the reservoir. Upper scales, cm. H_2O. Lower scales, mm. Hg.

before it was circulating. In experiments performed in this way, the cardiac effects were much more gradual in onset, and the preparation lasted for about 2 hours before the appearance of œdema. Fig. 7 is taken from one of these, in which a little of the blood was kept and added to the reservoir later; the effect on the heart and pulmonary vessels is well marked. In Fig. 8 is recorded a similar experiment, in which the effects of the reserve blood and of histamine are seen side by side; there is a great similarity between them, and this is emphasized by the additional record of tracheal pressure.

The method of recording tracheal pressure was suggested by Dr H. P. Gilding. The pump was of a type similar to Starling's "ideal" pump [Starling, 1926], such that "expiration" took place through a valve, which was automatically closed during inflation. The record (water manometer, float recorder) therefore indicates the pressure required to inflate the lungs with a given volume of air; this will be greater during bronchial constriction, and less during bronchial dilatation, and it may be affected in other ways.

Following the moderate rise in tracheal pressure on addition of histamine, the large and abrupt rise was almost certainly due to the onset of pulmonary œdema; at the point marked there was a sudden and copious flow of fluid from the tracheal cannula. Also, the onset was so rapid and followed so closely upon the administration of histamine, that there can be little doubt of this being the cause in this experiment. The subsequent fall in tracheal pressure followed upon the addition of adrenaline.

Discussion.

The first series of experiments requires no comment. The discussion of the results of the second series will be facilitated by stating the main conclusion at once, namely, that from the time it is shed, the blood develops a toxicity which is manifested in two ways, by an action on the cardiac muscle and by an action on the pulmonary circulation; both of these contribute to the production of pulmonary œdema.

1. *The heart muscle.* Patterson and Starling [1914] stated that "when failure occurs under a maximal load, either the right or the left side of the heart may fail before the other side." As an index of the former they took a high venous pressure; of the latter, pulmonary œdema. This view of œdema as being obstructive in origin prevails in the literature, and is supported by experiments in which the heart has been subjected to damage of the left side, or to an excessive load. Lambert and Gremels [1926], however, observed no signs of cardiac failure in their experiments on the dog heart-lung.

In interpreting the results of the present work, a rising left auricular and falling aortic pressure can only indicate inability of the left side of the heart to pass on blood entering from the lungs, while a steady high left auricular pressure must mean an output maintained with difficulty. Whether or not the condition of the right heart is affected, its output in

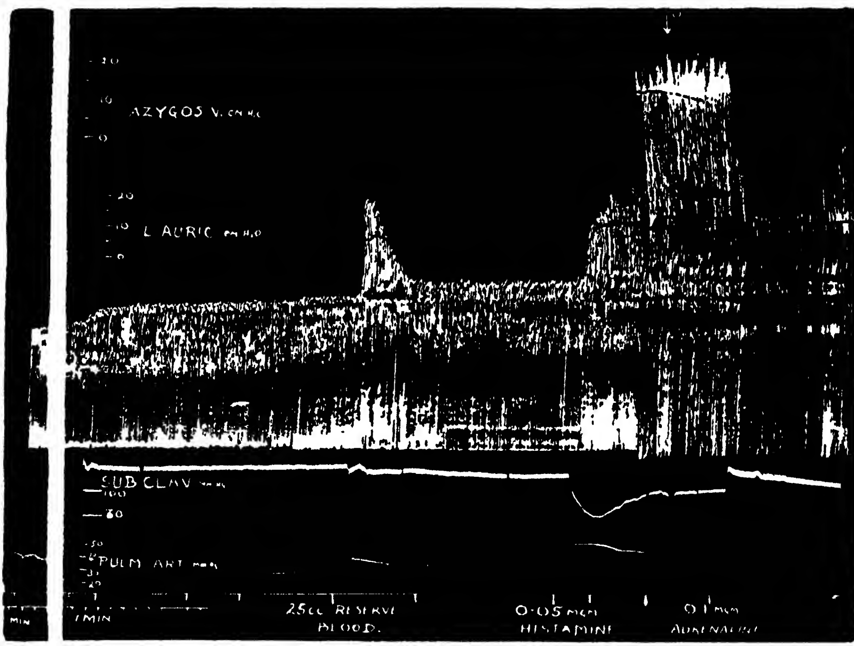

Fig. 8. Complete experiment, showing effects of reserve blood (same sample as already in reservoir, but kept standing for 22 min.), histamine, and adrenaline. P.O. ≡ pulmonary œdema. Signals: output 102 c.c. per min.; output 107 c.c. per min.; output 112 c.c. per min.; 25 c.c. reserve blood; output 100 c.c. per min.; 0·05 mgm. histamine; output 45 c.c. per min.; output 67 c.c. per min.; 0·1 mgm. adrenaline. After "P.O." superior vena cava pressure of no significance.

the first case is greater than that of the left, and in the second case is sufficient to supply enough blood to keep the left ventricle adequately dilated. In this sense, we may speak of a "relative incompetence" of the left side of the heart, and as the inflow and resistance are low and constant, the occurrence of this is referred to the condition of the cardiac muscle. The incompetence, as indicated by the left auricular pressure, was slowly progressive in the later experiments, but came on rapidly in the earlier ones; in these, the heart then either failed entirely, or

temporary equilibrium was attained, being preceded in some cases by a great measure of recovery.

In an attempt to correlate the height of the left auricular pressure with the incidence of œdema, eleven experiments were arbitrarily divided into two groups, those in which œdema developed within one hour, and those in which it did not. With times from the establishment of the preparation as abscissæ, and pressures as ordinates, two areas were plotted, each containing all the auricular pressures of one group up to the end of an hour. Although the pressures of the first group were in general higher than those of the second, the two areas overlapped. Moreover, within the first group the preparations with the highest pressures were not always those which developed œdema most rapidly, and preparations in the second group developed œdema in course of time. It seemed, therefore, that while a damming up of blood in the lungs predisposed to œdema, some other factor was at work.

A rising pressure in the superior vena cava has been taken as indicating a failure of the right heart to propel through the lungs the blood it is receiving. The experiments do not indicate whether this is due to feebleness of the muscle, augmentation of inflow from the coronary circulation, or increased obstruction in the pulmonary circulation. The inflow from the reservoir at any moment could be calculated approximately from the superior vena cava pressure; this calculated inflow agreed fairly well with the observed output when this was taken. In different preparations, the same left auricular pressure may be combined with varying rates of flow, and it was thought possible that the amount of blood passing might be an additional factor in the causation of œdema, but this is not the case. Areas plotted as described above, but with some combination of left auricular pressure and flow as ordinates instead of simple left auricular pressures, showed no greater separation than before. The degree of right heart failure, therefore, seems to have little to do with the question.

2. *The pulmonary circulation.* Most of the relevant tracings have been transferred to squared paper, and the pressures plotted in the same units (mm. H_2O). These show conclusively that the left auricular and pulmonary arterial pressures change independently of one another; one may rise while the other is falling, quite spontaneously. When kept blood is added to the venous reservoir, the rise in pulmonary pressure is much too large to be accounted for by a damming back of blood from the left heart. In Fig. 7 it is five times as great as the rise in left auricular pressure; in the same experiment the inflow was increased to give the same rise in left auricular pressure, and the rise in pulmonary pressure

was almost identical with it. Such findings suggest strongly that the blood has a separate action on the vessels of the lung. Moreover, by again grouping the experiments into those giving and those not giving œdema in an hour, and plotting areas as before, it was found that these separated completely after the first 25 minutes, if the products of left auricular and pulmonary arterial pressures were taken as ordinates instead of left auricular pressures alone. With pulmonary pressures alone as ordinates, the areas showed no separation whatever (Fig. 9). This means that a high pulmonary pressure reinforces a high left auricular pressure in the production of œdema, and is in accordance with the impression gained from examination of the individual tracings. The probable length of an experiment can be predicted by watching the progress of these two pressures and the relation between them.

How a high pulmonary arterial pressure can predispose to œdema is difficult to say. If it is due to constriction of the arterioles, the capillary pressure ought not to be unduly affected; if it is due to capillary constriction, it is not easy to explain the congested appearance of the lungs just prior to the onset of œdema. It may be that a simultaneous arteriole constriction and capillary dilatation occur; this is supported by the fact that histamine, which might be supposed to have some such action, gives a very similar tracing to kept blood. In the experiment illustrated in Fig. 8, this similarity is seen to extend to the effect on tracheal pressure (probably a constriction of the bronchioles) and to the production of œdema. Mautner and Pick [1929] state that, in the dog, histamine causes constriction of the pulmonary veins.

The double action of adrenaline cannot be explained without further experiments. The dose which gives a fall in pulmonary pressure in Fig. 4 has been observed to give a rise upon other occasions. The remaining pressures are altered in the same way in both cases, emphasizing the fact that the pulmonary arterial pressure is largely independent of cardiac events. Blood, even if old, which has had adrenaline added to it, has the same effect as the latter.

3. *The coronary circulation.* No measurements of coronary flow have been made, but there is little doubt that, when this is done, the general scheme as here presented will have to be modified. Lambert and Gremels [1926] observed a steady increase throughout their experiments, and attributed the rise in pulmonary pressure partly to this and partly to obstruction from the œdema itself. Dilatation of the coronary arteries could be made, theoretically, to account for many of the phenomena observed, but this would involve the assumption of a coronary flow so

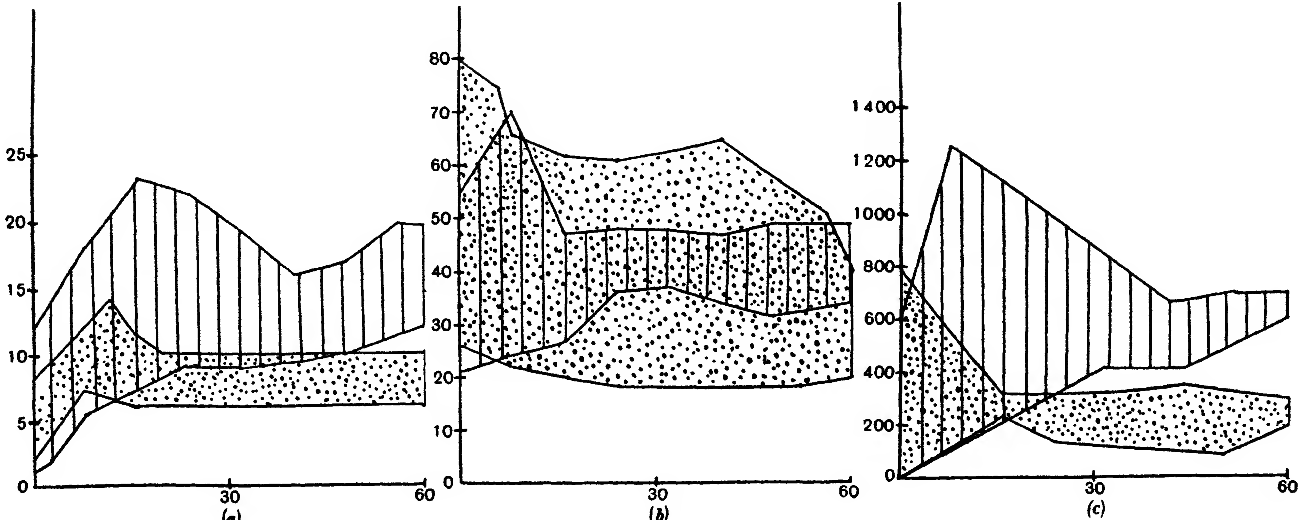

Fig. 9. Shaded area includes preparations developing œdema within one hour. Stippled area includes preparations not developing œdema within one hour. Abcissæ: time in minutes. Ordinates: (*a*) Left auricular pressures in cm. H_2O; (*b*) pulmonary arterial pressures in cm. H_2O; (*c*) ordinate of (*a*) × ordinate of (*b*).

large as to embarrass both sides of the heart. Also, adrenaline, which is known to dilate the coronary arteries, has a very different action from that of kept blood (Fig. 4), while the improvement of the heart after glucose (Fig. 5) points to the muscle rather than the vessels as having been affected.

4. *The ventilation of the lungs.* It is probable that the rise in tracheal pressure (Fig. 8) after the addition of kept blood and histamine is due to a constriction of the bronchioles; this is further suggested by the fall after adrenaline, which is known to cause broncho-dilatation. Simultaneous circulatory changes, however, may play some part, particularly back-pressure from the left auricle. When very fresh blood was used, and deterioration of the preparation gradual, the rise in tracheal pressure was also gradual, and it seems likely that this would be due to congestion or to the accumulation of fluid in the alveoli; the second rise after histamine, in Fig. 8, was almost certainly due to the fluid which appeared a few minutes later in the tracheal cannula. A possible effect of vascular conditions upon pulmonary ventilation has been observed in man by Campbell and Poulton [1927].

5. *The blood.* In the earlier experiments, when the blood stood while the preparation was being made, the pressure changes were observed very early and in a marked degree; in many cases they returned towards the normal after about 10 minutes. When the blood was used immediately after shedding, the pressure changes were gradual and progressive for about 2 hours; but transient, although marked, changes (Figs. 7, 8) could be superimposed upon the main tracing by the addition of a small quantity of the identical blood which had been kept for half an hour and not used in the circulation. It is apparent, therefore, that from the time it is shed the blood progressively becomes toxic, and this toxicity is partially inhibited by passage through the heart-lung preparation; the length of an experiment seems to depend upon which process gains ascendancy at the beginning. Occasionally, freshly shed blood had a positive beneficial action on the heart, while still increasing the pulmonary pressure; the effect was exactly the same as that of adrenaline, and was attributed to the presence of this substance in the blood, which had been obtained in each case by abdominal pressure from a cat already bled.

Freund [1920], working on the intact animal, described a transient depressor action of defibrinated blood, followed by the development of a pressor action. He postulated "Frühgifte" and "Spätgifte" to account for these. Zipf [1931] has identified the depressor component of Freund's Frühgifte with adenylic acid, which he has shown to be present in freshly

defibrinated blood; he was unable to demonstrate either depressor activity or adenylic acid in oxalated or heparinized blood. He quotes several observers, in addition to himself, who have reported the absence of histamine in freshly shed blood. Phemister and Handy [1927] described a depressor, giving way to a pressor, action of shed heparinized blood; neither of these appeared in the absence of trauma, and they related them to the occurrence of small and large degrees of haemolysis respectively. Histamine would not account for their results. Blood vessels rapidly acquired the power of resisting the dilator effect. Eichholtz and Verney [1924] demonstrated that "Vasotonins appearing spontaneously in defibrinated blood are inactivated during the passage of this blood through a heart-lung preparation."

Apart from the power of the tissues to overcome the toxic action, the results recorded in this paper cannot be correlated in detail with those of the observers just quoted. The blood has been subjected to no more trauma than that involved in collecting it in a beaker and transferring it to the venous reservoir, while its activity increases simply on standing. Its effects are unlike those of adenylic acid, which, for instance, causes relaxation of the bronchioles [Bennet and Drury, 1931], and bear a striking resemblance to those of histamine. Whether its action on the systemic vessels is pressor or otherwise has yet to be determined, and the question must be left open. Microscopic sections confirm the findings of Lambert and Gremels [1926] as to capillary damage in the lungs.

Conclusions.

1. The pulmonary œdema which occurs in the cat heart-lung preparation is primarily due to a toxic activity of the blood which is used. This blood is rendered incoagulable by the intravenous injection of Chicago blue, and develops its toxic properties simply upon standing. If used immediately, the onset of œdema is delayed.

2. Its action is on the heart, pulmonary circulation, and probably bronchial muscle. In the heart it causes weakening of the muscle, acting predominantly upon the left side which is relatively incompetent (in a sense defined), thereby producing an increased intracapillary pressure in the lungs. In the pulmonary circulation it causes a rise in arterial pressure; the significance of this may be only as an index to a simultaneous toxic action upon the capillaries.

3. Almost identical reactions are provoked by histamine. The effects do not correspond with those reported by others for adenylic acid.

4. Adrenaline may produce either a rise or fall in the pulmonary arterial pressure, but its cardiac action is constant and the reverse of that of shed blood and histamine. Glucose has the same cardiac effect as adrenaline.

5. The passage of the blood through the heart-lung preparation reduces its toxicity.

I wish to thank Prof. C. Lovatt Evans and Dr H. P. Gilding for advice throughout the course of this work.

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INHIBITION OF THE ANAPHYLACTIC REACTION BY CONGO RED.

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In a recent paper Nicolaeff and Goldberg [1930] showed that the anaphylactic reaction in guinea-pigs could be inhibited by a number of substances including congo red. The intraperitoneal injection of congo red into sensitized animals 10–30 minutes before the administration of the specific antigen prevented the subsequent development of the anaphylactic reaction. In view of the fact that previous observers [Klopstock, 1924; Hanzlick and Butt, 1928; Gordon, 1930] have shown that the dye interferes with a number of immunological processes, it appeared of interest to investigate in greater detail the factors involved in the anaphylactic interference phenomenon.

Experimental.

Immature guinea-pigs weighing about 150 g. were sensitized by the subcutaneous injection of 0·2 c.c. of rabbit serum. After an interval of 18 days or more, experiments were performed on 58 sensitized animals comprising 33 animals injected with congo red and 25 controls. The former were injected into the peritoneum with 5 c.c. of a 1 p.c. solution of congo red in normal saline. After an interval of half an hour 10 c.c. of rabbit serum was again injected intraperitoneally. The results obtained are set out in Table I. It will be noted that the mortality in the animals injected with congo red was much less marked than in the control group; only 1 of the guinea-pigs died within 2 hours and 24 of the animals showed little or no effect, while 20 out of the control group died within 2 hours and the remaining animals either died subsequently (3) or showed severe symptoms (2). These experiments clearly indicate that congo red inhibits or decreases the anaphylactic reaction.

TABLE I. To show the effects of congo red on the anaphylactic reaction in guinea-pigs.

Animals injected with congo red.

		Died		Symptoms	
Exp. No.	Animals used	Within 2 hr.	Next day	Severe	Mild or nil
1	5	—	—	—	5
2	5	—	—	—	5
3	3	—	2	1	—
4	4	—	1	—	3
5	3	—	1	—	2
6	3	—	1	—	2
7	10	1	2	—	7
Total	33	1	7	1	24

Control animals.

		Died		Symptoms	
Exp. No.	Animals used	Within 2 hr.	Next day	Severe	Mild or nil
1	5	5	—	—	—
2	2	2	—	—	—
3	2	2	—	—	—
4	3	1	—	2	—
5	2	2	—	—	—
6	1	1	—	—	—
7	10	7	3	—	—
Total	25	20	3	2	—

In order to investigate the factors responsible for this inhibition, experiments on the isolated uterus of sensitized animals were performed. The horns were suspended in 100 c.c. of Ringer-Locke solution; temperature (37° C.) and oxygen bubbling were maintained constant and records were taken on a smoked drum with lightly balanced lever. After the base line had become constant, 4 c.c. of a 1 p.c. solution of congo red were added to the bath (giving a concentration of 1 in 2500); an interval of at least half an hour was allowed to elapse and rabbit serum was then added (usually 0·01 c.c.). No effect was obtained, although the control horn kept under similar conditions gave a positive anaphylactic reaction. On changing the solution and allowing a suitable interval to elapse, the addition of rabbit serum to the bath now brought about a contraction. Fig. 1 illustrates the effects observed on adding rabbit serum to the bath in the presence and subsequent absence of congo red. Between the two tracings the solution was changed twice and an interval of about 1 hour elapsed. It is therefore seen that the *in vitro* congo red inhibition is reversible in nature.

It appeared possible that the presence of congo red might interfere with the contractility of the uterine muscle, and the response of the uterus to a number of drugs was therefore investigated. It was found that the presence of the usual amounts of congo red in the solution did not in any way interfere with the reaction of the muscle to pituitrin. The

response to histamine was also investigated. Repeated additions of this drug are followed by a decrease in the height of the contraction (the solution being changed after each addition), but as far as could be observed congo red did not cause any decrease in the contraction. Nor was the inhibitory action of adrenaline interfered with by the dye. It must therefore be concluded that the action of congo red is not dependent on an interference with the contractility of the uterine muscle.

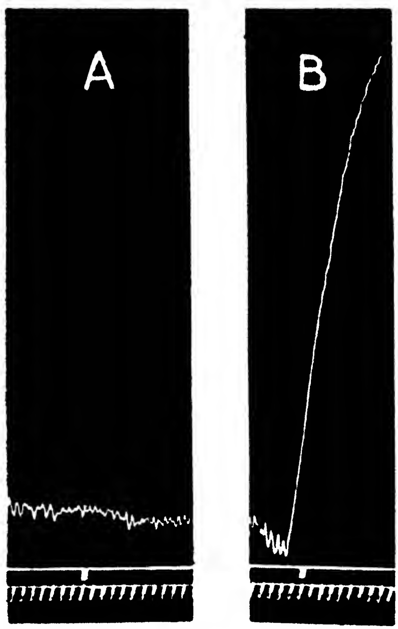

Fig. 1. To show effect of congo red on the anaphylactic response of the isolated guinea-pig uterus. A. Effect of 0·01 c.c. rabbit serum in presence of congo red. B. Effect of 0·01 c.c. rabbit serum after changing the solution. Time intervals = 1 min.

If it be assumed, as at present accepted [Dale, 1929], that the anaphylactic phenomenon is due to an antigen-antibody reaction inside the cell, the experiments described above are capable of two interpretations. Firstly that congo red interferes with the entrance of the antigen into the cell, or secondly that it interferes with the antigen-antibody reaction. A series of experiments was designed to test the effect of congo red on a number of other antigen-antibody reactions.

Ox serum was injected on several successive occasions into rabbits and a precipitating antiserum was thus prepared. To tubes containing

1 c.c. of this antiserum (rabbit serum) were added 0·1, 0·2 and 0·3 c.c. of a 1 p.c. solution of congo red in normal saline. The mixtures were allowed to stand on the bench for half an hour and ox serum was then added to each of the tubes in amounts of 0·2, 0·1, 0·02, 0·01 and 0·005 c.c. Similar control experiments without congo red showed that congo red did not interfere with the precipitin reaction, for precipitation occurred at the

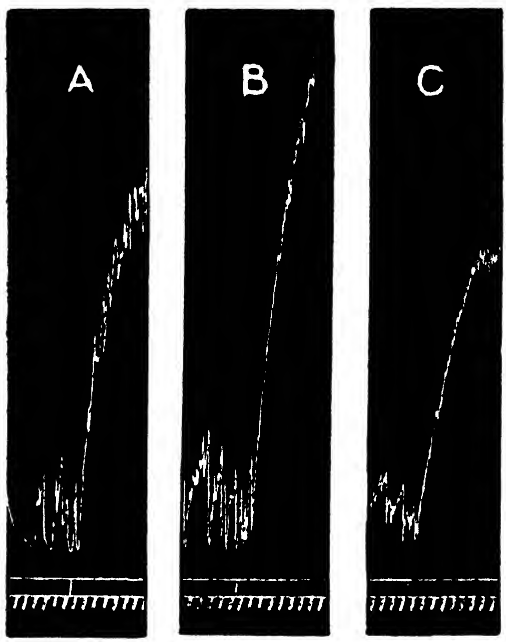

Fig. 2. To show the effect of congo red on the response of the isolated guinea-pig uterus to pituitrin. A. Effect of 0·05 U. pituitrin. No congo red present. B. Effect of 0·05 U. pituitrin in presence of congo red. C. Effect of 0·05 U. pituitrin. No congo red present. Time intervals = 1 min.

same titre in both sets of tubes. Similar experiments were performed with agglutinating sera (*B. typhosus*, *B. paratyphosus* A and *B. paratyphosus* B) and in no case did congo red cause any interference with the agglutinating reaction which is again an antigen-antibody reaction.

Further confirmation of the view that congo red does not interfere with the antigen-antibody reaction was afforded by an *in vivo* experiment. Twelve rabbits were used and to six of them 10 c.c. of a 1 p.c. solution of congo red in normal saline were injected intravenously daily for 6 days. The twelve animals were now injected intravenously with

2 c.c. of a goat serum which had a marked agglutinating action on the rabbit's red blood corpuscles. All the rabbits were very rapidly affected and died in periods varying from 5 minutes to several hours. The histological sections showed that the capillaries were full of agglutinated red blood corpuscles.

The rabbits which had been injected with congo red showed no different reactions from those which had not been treated with the dye. When tested *in vitro* the goat serum had the same agglutinating action on rabbits' red blood corpuscles in the presence of congo red.

It may therefore be concluded that congo red does not interfere with the union of various antigens or antibodies.

Discussion.

The experiments described in the early part of the paper clearly demonstrate that congo red can inhibit the anaphylactic reaction in guinea-pigs, for the injection of antigen into animals treated with congo red produced no effect in the majority of cases. A similar effect can be observed in experiments on the isolated uterus of sensitized animals; it has been shown that the addition of congo red to the solution prevents the subsequent reaction to the antigen. The presence of congo red in the solution is actually necessary for this inhibitory phenomenon, for if the solution is changed the reactivity of the uterine muscle gradually returns and the anaphylactic response can, after a suitable interval, again be obtained. The *in vitro* inhibitory action of congo red is therefore definitely reversible in nature.

The failure of the antigen to cause contraction of the muscle appears to indicate that the intracellular antigen-antibody reaction responsible for the anaphylactic phenomenon is interfered with by the dye. As it has been shown that the contractility of the muscle to pituitrin and histamine remains unimpaired, it seems likely, therefore, that either (1) the diffusion of antigen into the cell is not taking place, or (2) the formation of the antigen-antibody complex is inhibited. This latter suggestion appears rather unlikely in view of the failure of congo red to interfere in any way with a number of antigen-antibody reactions. Similar evidence is afforded by the experiments of Gordon [1930], who showed that congo red did not prevent the adsorption of hæmolytic immune body on to red blood corpuscles. It seems likely, therefore, that congo red actually interferes with the entrance of antigen into the cell. On changing the solution the dye is removed and the usual reaction can again take place.

It appears probable that the *in vivo* inhibitory phenomenon is dependent on a reaction of the nature described above for the *in vitro* effects, and that the concentration of dye sufficient to interfere with the entrance of antigen into the cell actually occurs in the animal's body fluids. The possibility of this occurrence is strongly supported by the finding of Bolton [1921] that congo red is very rapidly absorbed into the blood stream when injected into the peritoneal cavity. Whether congo red can interfere with the absorption of the antigen from the peritoneum and thus produce an additional factor in preventing the development of the anaphylactic reaction has so far not been considered here, and the data at our disposal do not make it possible to eliminate this explanation.

Summary.

The intraperitoneal injection of congo red shortly before the injection of antigen interferes with the subsequent development of the anaphylactic reaction in sensitized guinea-pigs.

Congo red can prevent the *in vitro* anaphylactic reaction of the guinea-pig uterus.

Congo red does not interfere with the contractility or reactivity of the uterine muscle.

Congo red does not interfere with a number of *in vitro* and *in vivo* antigen-antibody reactions.

It is suggested that the dye interferes with the entrance of antigen into the cell.

We should like to express our indebtedness to the Medical Research Council for a grant in aid to one of us (J. G.).

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THE CARBOHYDRATE METABOLISM OF THE ISOLATED HEART OF THE FROG.

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I. Changes in carbohydrate content during perfusion.

The authors noted in a previous paper [1931] that, after frogs' hearts had been perfused for 6 hours with Ringer's fluid, the average content of reducing substances was 1·57 p.c., which was higher than the average obtained with unperfused hearts (1·42 p.c.); moreover, during perfusion sugar was excreted equal to 0·14 p.c. of the heart weight. Our figures therefore showed a clear increase in reducing substances during perfusion. We repeated these observations on a fresh series of frogs, using the same methods as before. The only change was that a Barcroft apparatus was used with a large bulb in which three hearts could be placed. This represented an important saving in time. In addition to measuring the changes in carbohydrate content, etc., in hearts perfused with Ringer's fluid, we also investigated hearts perfused for 6 hours with fluids that depressed the mechanical activity of the isolated heart, *e.g.* calcium-poor Ringer's fluid and Ringer's fluid containing 0·9 molar ethyl alcohol. The carbohydrate content of the frogs' hearts varied, both as regards the season of the year and the duration of captivity, and therefore control estimations on unperfused hearts were made throughout the course of the experiments. The values obtained at different periods are shown in Table I. The effects of perfusion on the

Table I. Total reducing substances in unperfused hearts.

Period	No. of hearts	Average weight in g.	Average p.c. of reducing substances
(*a*) April, 1931–July, 1931	24	0·147	1·228
(*b*) Oct. 1931–Nov. 1931	20	0·165	1·197
(*c*) Dec. 1931–Jan. 1932	10	0·124	1·553

[1] Carnegie Teaching Fellow. In receipt of part-time grant from the Medical Research Council.

hearts are shown in Table II. In the experiments with calcium deficiency and alcohol the perfusion fluid was adjusted so that the heart's activity was reduced until it just maintained a circulation. Table II shows that

TABLE II.

Date (1931)	Nature of perfusion fluid	Duration of perfusion in hours	No. of hearts	Average heart wt. in g.	Reducing substance in p.c. (a) Perfused hearts	(b) Controls	Sugar excreted per heart in mg.	Nitrogen excreted per heart in mg.	Oxygen used per heart in c.c.	R.Q.	Loss or gain of reducing substance after perfusion in mg. per heart: Loss	Gain
April–June	Normal Ringer's fluid	6	11	0·151	0·870	1·228	0·055	0·063	1·26	0·89	0·48	—
Dec.	do.	24	9	0·120	0·966	1·553	—	—	—	—	<0·70	—
May	Ringer containing about 0·004 p.c. $CaCl_2$	6	15	0·169	1·417	1·228	0·024	0 052	0·44	0·87	—	0·34
Oct.	do.	6	15	0·130	1·330	1·197	0·13	—	0·35	0·88	—	0·30
Oct.	Ringer containing ethyl alcohol 0·9 molar	6	9	0·152	0·969	1·197	0·29	0·031	0·45	0·85	0·06	—
Oct.	Ringer plus 5 p.c. inositol	6	9	0·147	0·685	1·197	0·26	0·023	0·60	0·86	0 50	—

perfusion for 6 hours with Ringer's fluid caused a loss of reducing substances. In our previous experiments, already mentioned, we found an increase under these conditions. The loss of 0·48 mg. per heart shown by the averages in the present experiments was largely due to unusually low values obtained with three hearts in one experiment. The remaining eight hearts showed an average loss of only 0·27 mg. reducing substance per heart. Hearts suffering from lack of oxygen rapidly turn carbohydrate into lactic acid, hence any error in the technique of perfusion will tend to produce a loss of reducing substance; we believe that the loss in this case was due in part to an experimental error, particularly because the 24-hour experiments showed only a slightly greater loss of reducing substances.

The experiments with inositol were made to see if this substance spared the carbohydrate content of the heart. The hearts were definitely injured by the presence of inositol as is shown by their low oxygen consumption, hence the carbohydrate loss in this case has little significance, except that it shows that inositol does not spare the carbohydrates in the heart.

The hearts perfused with solutions that reduced their mechanical activity and oxygen consumption show either a gain or no significant loss of carbohydrate as a result of perfusion. A gain in reducing substance during 6 hours' perfusion is shown even if variations in the

carbohydrate content of the controls be ignored and general averages of all our experiments be taken. From Jan. 1930 to Jan. 1932, 90 estimations were made of the total reducing substance in fresh hearts, and the average value of these estimations was 1·32 p.c. total reducing substance. In the same period 28 hearts perfused with normal Ringer's fluid for 6 hours gave an average total reducing substance content of 1·30 p.c., and 39 hearts perfused for 6 hours with Ringer's fluid containing depressants gave an average of 1·28 p.c. The average sugar excretion in both cases was equivalent to about 0·1 p.c. of the heart weight, and hence the reducing substance in the heart and fluid after perfusion in the two cases averaged 1·40 and 1·38 p.c. values, which are definitely above the control value of 1·32 p.c.

Another method of showing our results is to calculate the reducing substance recovered as the percentage of the reducing substance present in the controls. The two general averages just given for 6 hours' perfusion work out at 105 and 106 p.c. respectively. Table III shows a

TABLE III. Carbohydrate and oxygen usage of hearts.

Experimental conditions	Duration of perfusion in hours	Total reducing substance as mg. per g. Present in controls	Total reducing substance as mg. per g. Present in perfused heart	Sugar excreted mg. per g. heart	Total reducing substance recovered expressed as p.c. of that present in controls	Oxygen used c.c. per g. heart
II. Previous exps. [Clark *et al.*, 1931]:						
Perfused Ringer's fluid	6	14·2	15·7	1·35	120	7·6
,, ,, ,,	24	14·2	7·3	3·0	72·5	18·0
Perfused Ringer-serum mixture	6	14·2	10·5	0	74	11·4
	24	14·2	4·8	0	34	22·8
II. Present exps.:						
Perfused Ringer's fluid	6	12·28	8·70	0·36	74	8·3
,, ,, ,,	24	15·53	9·66	—	62	—
Perfused calcium-poor Ringer's fluid						
1st series	6	12·28	14·17	0·14	116	2·62
2nd series		11·97	13·30	1·0	119	2·69
Perfused ethyl alcohol 0·9 molar	6	11·97	9·69	1·9	96	2·96

series of values of this kind calculated for both the present experiments and certain experiments recorded in our previous paper. In order to make the figures comparable they have all been calculated on the basis of carbohydrate and oxygen used per g. of heart.

The results recorded in Table III are shown as a graph in Fig. 1. The figures show a considerable scatter, but taken as a whole they indicate that during perfusion a quantity of reducing substance appears from some unknown source which corresponds to 20 p.c. of the quantity present in the controls or to 0·4 mg. in a heart weighing 0·15 g.

Similar conclusions have been made by other workers. Wertheimer [1930] found no certain change in either the glycogen or total carbohydrate contents of heart strips of the frog after isolation for many hours. Hines, Katz and Long [1925] showed that the normal glycogen content

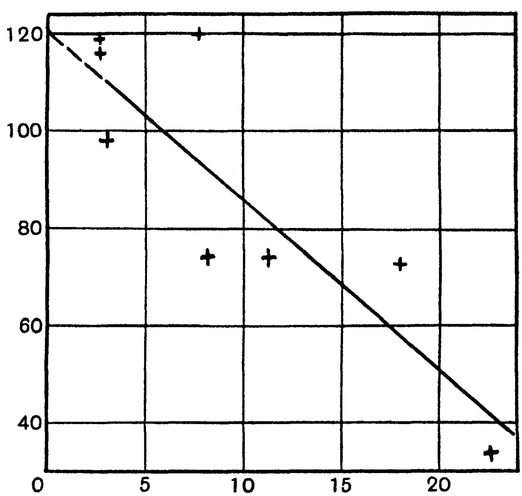

Fig. 1. Relation between oxygen consumption and carbohydrate loss in the perfused frog heart. Figures taken from Table III. Abscissa: total oxygen consumption in c.c. per g. Ordinate: total reducing substance recovered expressed as percentage of values found in appropriate controls.

of the heart muscle of cats was 0·138 p.c., whereas the lactic acid maximum in rigor mortis was 0·32 p.c. Boyland [1928] studied the production of lactic acid in minced heart muscle of the tortoise and of the frog incubated with alkaline phosphate mixture. He concluded that a quantity of lactic acid corresponding to between 0·1 and 0·2 p.c. of the muscle weight must be produced from a source other than the reducing carbohydrates. This agrees fairly well with the excess of reducing substances found by us, which corresponded to about 0·3 p.c. of the heart weight. Boyland considered that inositol was a likely source of the excess of lactic acid. He found 0·37 p.c. inositol in pig's heart. He found that the inositol in the heart decreased on incubation with phos-

phate mixture, and that addition of inositol to such a system increased the amount of lactic acid formed.

Our conclusion that a certain quantity of reducing substance is formed from some non-reducing source agrees therefore with the results obtained by other workers. One obvious possible source of error was the incomplete hydrolysis in the estimation of total reducing substances. The hearts were hydrolysed for 3–4 hours, and the following experiment showed that prolongation of the time of hydrolysis did not increase the total reducing substances.

Exp. Eight frog hearts, total weight 0·626 g., were hydrolysed for 24 hours and the total reducing substances were estimated in fractions at varying times. (The amount found was twice the usual value, but the frogs used were from a different source.)

Duration of hydrolysis in hours	3	5	7	24
Total reducing substance found as p.c. of moist weight	2·86	2·83	2·83	2·44

Exclusion of this source of error left the following problems to be investigated:

(*a*) The possibility of the presence of non-carbohydrate reducing substances which alter in amount during perfusion.

(*b*) Possible variation in the amount of lactic acid formed.

(*c*) The possibility of inositol being converted into a reducing substance.

(*a*) *The nature of the total reducing substance.*

We have previously shown [1931] that the substances included in our estimate of total reducing substance or total carbohydrate do not include creatine, the most obvious non-sugar likely to be included. Our results showing the apparent production of reducing substance during perfusion of the heart, however, rendered it necessary to examine more closely the nature of the substances estimated. For this purpose we used the fermentation method described by Somogyi [1927]. It was found that in fresh non-perfused hearts about 90 p.c. of the total reducing substance was fermentable, whereas pure glucose under similar conditions was completely fermented. In other words, of the 1·50 g. total reducing substance normally present in 100 g. of heart, 0·150 g. was non-fermentable. After perfusion for 24 hours the heart still contained 0·144 p.c. of non-fermentable reducing substance. Hence, if we are to consider only fermentable reducing substances, the figures for total carbohydrate must be reduced by an average amount of 0·150 p.c., but as the correction is the same for perfused and non-perfused hearts, all conclusions founded on the original figures are unaffected.

Although the presence of this non-fermentable reducing substance in the heart does not affect our main conclusions, it may possibly help to explain the curious fact that we rarely found a perfused heart to contain much less than 0·40 p.c. of total reducing substance, even under conditions such as anaerobiosis with alkaline perfusing fluid, which ought to produce complete exhaustion.

Rimington [1931] found that a number of proteins, on hydrolysis, yielded 3–4 p.c. of a trisaccharide which on further hydrolysis gave 2 mol. mannose (fermentable) and 1 mol. of glucosamine (non-fermentable). Our method of estimating total reducing substance involves a fairly prolonged preliminary hydrolysis, which might well split off and hydrolyse most, if not all, of this carbohydrate. Now the heart contains approximately 8 p.c. of protein which, on the basis of a 3·7 p.c. content of trisaccharide, would account for 0·296 g. reducing substance (calculated as glucose) per 100 g. of heart, and of this one-third or 0·10 g. would be non-fermentable.

The figures show that the residual reducing substance not fermented with yeast forms a small fraction of the total reducing substance, and that it remains unchanged during prolonged perfusion. The presence of this unknown material cannot account for the rise observed in total reducing substance during short periods of perfusion.

(*b*) *The formation of lactic acid.*

The authors have studied this subject and it will be dealt with in a later paper. The facts essential to the present discussion are that hearts, when perfused with good oxygenation, form scarcely any lactic acid, but that interference with the oxygen supply at once causes the breakdown of carbohydrate and the production of lactic acid. Any interference with the circulation of fluid through the heart may therefore cause a loss of carbohydrates. Hence the most probable error in our experiments is a conversion of carbohydrate to lactic acid during perfusion, and this would tend to increase the apparent loss of carbohydrate without increasing the oxygen consumption. If chemical treatment caused lactic acid formation more readily in the fresh heart than in the heart after perfusion this might explain the increase in carbohydrate content that we observed.

We found that the lactic acid content of fresh hearts frozen with carbon dioxide snow was 0·06 p.c., and treatment with acid at room temperature raised this value to 0·09 p.c. This value was the same in fresh hearts and in hearts after perfusion, and leaving hearts lying in

Ringer's fluid without oxygenation did not raise this value. We have therefore no reason to suppose that lactic acid formation during chemical treatment would alter the relative values of the carbohydrate content of the fresh and of the perfused hearts.

(c) *Inositol as a source of reducing material.*

Boyland [1928] suggested that inositol might act as a source of reducing substances. We found that it was present in the hearts both of mammals and skates.

The method used to estimate inositol in heart muscle was practically that described by Needham [1926]. Minced tissue was extracted for 24 hours with acetone, sufficient being used to give, with the water already present, a concentration of 70 p.c. acetone. The acetone was then removed *in vacuo* and the aqueous residue precipitated with lead acetate, and finally with basic lead acetate and a little ammonia (freshly prepared). The former precipitate was discarded, and the latter, which contained inositol, thoroughly decomposed with hydrogen sulphide. The clear filtrate obtained after removal of lead sulphide was concentrated to a few c.c. on the water bath. Inositol was precipitated by alcohol and ether, filtered off, dried, and weighed.

This method was applied to bullock's heart and to the heart of the skate, and their inositol contents were found to be respectively 0·10 p.c. and 0·073 p.c. of wet heart weight. We were unable to get any proof either direct or indirect of the conversion of inositol to hexoses. Various workers have described a stimulant action of inositol on the heart [Chevalier and Brissemoret (1908, rabbit's heart); Sachs, 1906; Hewitt and de Souza, 1921]. The effects recorded are, however, only slight.

Inositol added to perfusion fluid did not appear to have any carbohydrate sparing action on the heart in presence of oxygen (cf. Table I). This is inconclusive, because glucose has little action under such conditions, but the figures also show that no measurable conversion of inositol to sugar occurred. We found that glucose had a strong specific stimulant action on the heart of the frog exhausted by perfusion with alkaline fluid in the absence of oxygen, but inositol produced no benefit under these conditions. We cannot therefore produce any evidence in support of Boyland's suggestion.

II. The metabolism of the isolated frog's heart.

Table II shows the carbohydrate balance and oxygen consumption in five sets of experiments. In three of these no carbohydrate loss appeared, and in the other two cases the carbohydrate loss was 0·48 and

0·50 mg. This last figure corresponds to an oxygen consumption of $0·5 \times 0·75 = 0·38$ c.c., which is about 30 p.c. of the oxygen consumption recorded with Ringer's fluid. The figures with inositol are scarcely relevant as the hearts were partially poisoned. The respiratory quotients obtained all lay between 0·85 and 0·9, and therefore definitely indicated a considerable carbohydrate consumption. On the other hand the nitrogen excretion was in no case sufficient to account for more than a fraction of the oxygen consumption. Fig. 2 shows the general relation between nitrogen excretion and oxygen usage found in our present and previous experiments [Clark *et al.* 1931, Table III]. This indicates that the excretion of 0·6 mg. nitrogen is associated with 10 c.c. oxygen usage;

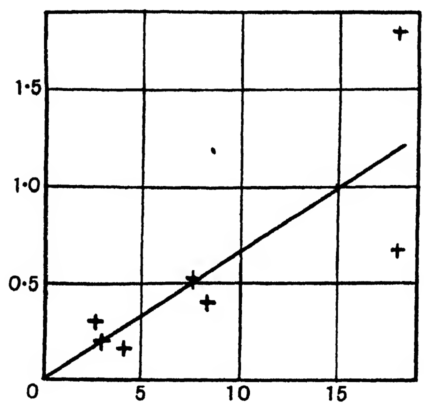

Fig. 2. Relation between oxygen consumption and nitrogen excretion. Figures from Table II and from Clark, Gaddie and Stewart [1931, Tables III and X]. Abscissa: oxygen consumption in c.c. per g. Ordinate: nitrogen excretion in mg. per g.

if the oxidation of material containing 1 mg. of nitrogen uses 6 c.c., then the oxidation of material containing 0·6 mg. uses 3·6 c.c. The nitrogen excretion observed can therefore account for about 36 p.c. of the oxygen used. Fig. 1 indicates the essential difficulty met with in our experiments, namely, that the carbohydrate content of the heart and the perfusion fluid together only falls to the level of the control values after an oxygen consumption of about 6 c.c. per g. of heart, a quantity which is used by a heart after about 4 hours' perfusion with normal Ringer's fluid. The only way to meet this difficulty and to balance the oxygen consumption with the metabolic changes is to assume the production during perfusion of reducing and fermentable substances amounting to about 20 p.c. of the initial content. If this is done an approximate balance

can be produced. Fig. 1 shows that, as a general average, the consumption of 10 c.c. of oxygen is associated with the disappearance of 40 p.c. of the carbohydrate in 1 g. of heart. The general average for our controls is 1·32 p.c. reducing substance or 13·2 mg. per g., and 40 p.c. of this is 5·28 mg. This corresponds to an oxygen consumption of 3·96 c.c. oxygen, or 39·6 p.c. of the oxygen consumed.

The nitrogenous and carbohydrate metabolism demonstrated can therefore account for 36 + 39·6 or 76 p.c. of the oxygen consumption observed, provided that we assume that during the first few hours' perfusion there is a production of reducing substance from non-reducing material. The figures thus obtained suggest an equal oxidation of protein and of carbohydrate. The figures for nitrogen excretion are, however, minimum figures, whereas any experimental errors leading to deficient oxidation would increase the carbohydrate consumption by causing lactic acid formation. Hence the evidence indicates that oxygen consumption due to protein metabolism is greater rather than less than the carbohydrate metabolism. If the metabolism were 60 p.c. protein and 40 p.c. carbohydrate, this would give a R.Q. of 0·88, and 32 experiments in which hearts were perfused with Ringer's solution for 6 hours gave an average value for the R.Q. of 0·87.

The obvious objection to the hypothesis outlined is that we have been unable to discover any probable source for the reducing substance that appears in such a mysterious manner. We believe that this will have to await further advances in our knowledge of carbohydrate metabolism, but have thought it desirable to publish these results because they indicate that the cardiac metabolism is not so completely unlike that of skeletal muscle as was suggested by our previous paper.

SUMMARY.

1. At least 90 p.c. of the total reducing substance found in the fresh heart is removed by fermentation. The quantity of non-fermentable reducing substance does not alter during perfusion.

2. After perfusion with calcium-poor Ringer the carbohydrate content of the perfused frog's heart is higher than that of the controls.

3. The evidence suggests that a quantity of carbohydrate equivalent to about 20 p.c. of the total amount found in the controls is produced during perfusion from some unknown source.

4. We have been unable to obtain any evidence to prove the consumption of inositol by the frog's heart.

5. The assumption that fermentable reducing substance is produced from a non-carbohydrate source makes it possible to account for 76 p.c. of the oxygen consumption observed. According to this hypothesis the carbohydrate consumption would amount to about 40 p.c. of the total metabolism, which would give an R.Q. of 0·88.

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THE ANAEROBIC ACTIVITY OF THE ISOLATED FROG'S HEART.

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A study of the literature regarding the effect of oxygen lack on the heart showed that different workers had obtained very contradictory results. Some authors stated that oxygen lack produced an immediate and progressive depression, whilst others found that isolated frog's heart could maintain fair activity for more than a day when deprived of oxygen.

Preliminary experiments showed us that the effects produced by oxygen lack on the isolated frog's heart depended on two independent variables, namely the amount of carbohydrate available for use by the heart and the reaction of the perfusion fluid. The experiments described below were designed to determine the influence of these two variables. It was found that, by adjustment of the two variable factors mentioned above, it was possible to reproduce almost all the widely varying effects of oxygen lack reported by previous workers.

We have shown [Clark, Gaddie and Stewart, 1931] that under aerobic conditions at least half the material metabolized by the isolated frog's heart is non-carbohydrate. The experiments described below indicate that under anaerobic conditions the only important source of energy available for the heart is the conversion of carbohydrate to lactic acid. We have therefore studied in detail the production of lactic acid by the isolated frog's heart.

Methods.

The method used for the estimation of lactic acid was one recommended by Dr P. Eggleton, and was founded on the usual oxidation to acetaldehyde by potassium permanganate in presence of sulphuric acid and manganous sulphate, absorption of the distilled aldehyde in bisulphate and titration of the bound bisulphite with iodine. The iodine was standardized every day by estimation of lithium lactate, and the method was

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periodically checked by estimation of lithium lactate, the iodine being then standardized by one of the usual methods. The actual recovery of lactic acid was found to be between 94 and 96 p.c. of that known to be present.

The following two methods were used for perfusion of the hearts:

Method 1. Perfusion of the whole frog's heart with cannulæ in the sinus and the aorta [cf. Clark, Gaddie and Stewart, 1931].

Method 2. Perfusion of the isolated ventricle with a cannula in the auriculo-ventricular opening, and arranged so that isochoric records could be taken when desired. (An apparatus of this type is illustrated by Clark [1927, Fig. 1].) We stirred the fluid in the cannula either with air or nitrogen, and in the latter case closed the cannula with a rubber stopper fitted with a jet for the escape of gas. The nitrogen was rendered oxygen-free by passing through a heated quartz tube containing copper filings.

The standard Ringer's fluid used had the following composition p.c.: NaCl 0·65, KCl 0·015, $CaCl_2$ 0·012, Na phosphate 0·02, *p*H 7·6. In the experiments with alkaline Ringer's fluid $NaHCO_3$ 0·05 p.c. was substituted for the phosphate, and when gas was bubbled through this the *p*H rose to about 8·5. No glucose was added except in those cases where its addition is mentioned.

(i) The lactic acid content of the fresh heart of the frog.

Table I shows figures given by various authors for the lactic acid content of the fresh hearts of various animals.

TABLE I. The lactic acid content of fresh hearts.

	Author		Lactic acid content p.c.
Tortoise and turtle	Redfield and Medearis [1926]	Ventricle	0·04
	Gemmell [1928]	Auricle	0·036
		Ventricle	0·026
	Boyland [1928]	Ventricle	0·22–0·31
Rabbit and cat	Schenk [1924]	Heart	0–0·07
	Katz and Long [1925]	Heart	0·028

We measured the lactic acid content of the excised frog's heart both in the fresh condition and after keeping, in order to discover how readily the lactic acid production could be induced.

Our results, which are shown in Table II, agree with those already quoted (except those of Boyland). Perfectly fresh frogs' hearts frozen

TABLE II. The lactic acid content of the isolated frog's heart.

	No. of exps.	Lactic acid p.c.
Fresh hearts frozen with CO_2 snow	3	0·06
Fresh hearts dropped into acid at room temp.	5	0·09
Hearts left soaking for 60 min. in Ringer at room temp. and then frozen	3	0·09
Hearts perfused 30 min. and then frozen	1	0·085
Hearts perfused 5 hours with oxygen and then frozen	3	0·09

immediately contained 0·06 p.c. lactic acid. Under most other conditions a value of 0·09 p.c. lactic acid was obtained, but values higher than this were not found except where the conditions were definitely anaerobic.

(ii) The lactic acid maximum of the hearts.

Table III shows the figures obtained by various authors for the lactic acid maxima of the hearts of various animals.

Table III. Maximum lactic acid content of heart.

		Lactic acid (p.c.)
Turtles' and tortoises' hearts:		
Turtle ventricle strip suspended in nitrogen		0·16
and stimulated to exhaustion. Redfield and	Auricle	0·137
Medearis [1926], Gemmell [1928]	Ventricle	0·141
Chloroform rigor. Arning [1927]	Ventricle	0·280
Incubation with alkaline phosphate. Boyland [1928]		0·49–0·61
Frog's heart:		
Chloroform rigor. Arning [1927]		0·143–0·325
Rabbits' and cats' hearts:		
Stimulated to exhaustion with asphyxia. Katz and Long [1925]		0·072
Rigor mortis. Hines, Katz and Long [1925]		0·23

Boyland's figures show the effects of incubation with alkaline phosphate and are higher than the rest, but the others show that in rigor the lactic acid of the heart does not rise above 0·28 p.c., and that the cold-blooded heart is arrested when the lactic acid content reaches about 0·14 p.c. These figures are less than half the corresponding figures found with skeletal muscle.

(iii) The production of lactic acid during aerobic activity.

Nagaya [1929] found that when frogs' hearts were perfused with a Straub's cannula demonstrable quantities of lactic acid were formed. After 75 min. perfusion he found about 0·11 p.c. of lactic acid in the heart, which was equivalent to about 0·2 mg., and between 1 and 2 mg. in the perfusion fluid. The total production of lactic acid corresponded to about 4 mg. per g. per hour. When the hearts were perfused with a double cannula only a trace of lactic acid could be demonstrated in either the hearts or the perfusion fluid.

These experiments show that the method of perfusion is of importance. A heart set up with cannulæ in the sinus and in the aorta and perfused with an oxygenated fluid receives a good supply of oxygen

and no lactic acid is produced. On the other hand when the heart pumps a small volume of fluid backwards and forwards, as is the case with the Straub's cannula, it is in reality suffering from a partial oxygen lack and demonstrable quantities of lactic acid are found.

Eismayer and Quincke [1930] used the isolated ventricle, working under conditions similar to our method 2. They found an excretion of about 0·4 mg. per g. per hour in the presence of oxygen. Wertheimer [1930, Table IX] used strips of ventricle and found that after 6 hours' isolation only traces of lactic acid were present in the strips, and that the amount in the surrounding fluid corresponded to a figure between 0·06 and 0·09 mg. per g. per hour.

The most probable reason for the wide differences in lactic acid production obtained by different workers is that their methods varied as regards the efficiency of the oxygenation of the heart.

We used two methods of perfusion and found that the lactic acid production was much less when there was a circulation of fluid (method 1) than when the fluid was pumped to and fro (method 2). With method 1 we obtained the following results: nineteen hearts were perfused for 20 hours, the total weight was 3·98 g., and the total lactic acid recovered from the heart and perfusion fluid together was 2·65 mg. This corresponds to a production of 0·03 mg. lactic acid per g. per hour, a value which is of the same order but less than that obtained by Wertheimer.

Method 2 gave higher figures. Three hearts perfused for 6 hours gave a lactic acid production of 0·22 mg. per g. per hour. This is intermediate between Wertheimer's and Eismayer and Quincke's figures, but is much smaller than those given by Nagaya.

The oxygen consumption of the isolated frog's heart working under average conditions is about 1 c.c. per g. per hour [Clark and White, 1928], an amount which is sufficient to oxidize 1·3 mg. of carbohydrate. The lactic acid production, even with method 2, is therefore only a small fraction of the total metabolism.

(iv) Lactic acid production under anaerobic conditions.

Nagaya [1929] measured the lactic acid production of frogs' hearts perfused with addition of KCN ($M/1000$) for periods of 45–90 min. The average of 18 experiments recorded by him is 10·3 mg. lactic acid per g. per hour. Eismayer and Quincke [1930] measured the lactic acid production of ventricles supplied with Ringer's fluid perfused with nitrogen, and their figures show a production of 4·1 mg. lactic acid per g.

per hour. Wertheimer [1930] measured the lactic acid production of ventricle strips in presence of NaCN ($M/2000$ to $M/5000$). The total of seven experiments in which the strips (total weight 0·393 g.) were stimulated showed 0·035 p.c. lactic acid in the heart strips, while from the perfusion fluid 0·85 mg. lactic acid was recovered.

This shows that most of the lactic acid formed passed out into the prefusion fluid. The lactic acid produced corresponded to about 4 mg. per g., but the duration of the experiments was not given, probably it was not much more than an hour.

We investigated the lactic acid production of ventricles perfused (method 2) with strongly alkaline Ringer's solution ($NaHCO_3$ 0·05 p.c., pH about 8·5) through which nitrogen was bubbled. Ten ventricles were perfused for 60 min., their total weight was 1·31 g., and the total lactic acid recovered was 0·98 mg. or 0·075 p.c.

The quantity of lactic acid found in these hearts was the same as in the control hearts, and this confirms Wertheimer's conclusion that lactic acid is rapidly excreted into the perfusion fluid. It will be shown later that this conclusion is only true when the perfusion fluid is alkaline. The quantity of lactic acid excreted into the perfusion fluid was as follows:

Duration of anaerobiosis in min.	10	20	40	60	80	120
No. of observations	6	6	4	3	5	4
Average lactic acid excretion in mg. per g. of ventricle	0·61	1·84	2·8	2·2	2·3	3·5

The lactic acid production under the above conditions was also estimated indirectly by measuring the loss of total carbohydrate in the hearts. In six experiments with ventricles rendered anaerobic by nitrogen perfusion for 120 min. the total reducing substance was found to be 0·73 p.c. The reducing substance in 20 controls measured at this period was found to be 1·23 p.c. This indicates a probable loss of 5 mg. of reducing substances per g. heart weight in 2 hours. The lactic acid estimations show an excretion of 3·5 mg. per g. in this period.

A few preliminary experiments were made with hearts subjected to a vacuum. Method 1 was used. Four hearts were put up at a time and the circulation was arranged from a common reservoir containing 200 c.c. fluid. The method was not very satisfactory because auriculo-ventricular block frequently occurred. The vacuum was maintained for 4 hours.

The following results were obtained: fifteen hearts (average weight 0·174 g.) were found to contain after exposure to the vacuum 0·77 p.c. total reducing substance. The corresponding average value in 20 control

hearts was 1·23 p.c. The calculated loss of reducing substance was 4·6 mg. per g. in 4 hours. The lactic acid recovered from the perfusion fluid was 0·95 mg. per heart = 5·45 mg. per g.

Fig. 1 shows the effect of anaerobiosis in alkaline sugar-free Ringer's fluid on the mechanical activity of the heart, and also the lactic acid production under these conditions. The lactic acid production per g. per hour is as follows: 1st hour, 3 mg.; 2nd hour, 1·3 mg.; 3rd and 4th hours, less than 0·5 mg.

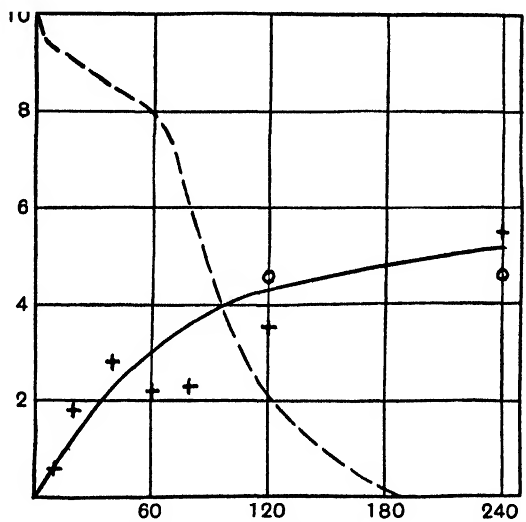

Fig. 1. The effect of anaerobiosis on the isolated frog's ventricle perfused with alkaline Ringer's fluid (pH 8·5). Abscissa: time in minutes. Dotted line: mechanical response (normal = 10). Continuous line: lactic acid production in mg. Crosses = direct estimations: circles = production as calculated from carbohydrate loss.

This rapid decrease in lactic acid production is accompanied by a corresponding decrease in the mechanical activity of the heart as estimated by the amplitude of the isometric response. The amplitude of the mechanical response of the heart during the first hour is, however, about 80 p.c. of the normal, and the lactic acid production that occurs during this period (3 mg.) is surprisingly low.

The data shown in Fig. 1 were for the most part obtained with the isolated ventricle, and we have no measurements of the oxygen consumption of the ventricle of *R. hung.* under these conditions; moreover our data do not show the actual work done by the ventricle. The results are therefore unsuitable for quantitative treatment in terms of energy,

but they certainly suggest that the energy liberated under anaerobic conditions is used more efficiently than that liberated under aerobic conditions.

(v) Lactic acid production in hearts perfused with neutral Ringer's fluid.

The effects produced by lack of oxygen on heart tissue are partially antagonized by the presence of alkali. This fact was noted by Martin [1905] in the case of strips of turtle's ventricle and by Drury and Andrus [1924] in the isolated dog's heart. We found that oxygen lack in hearts perfused with unbuffered Ringer's fluid produced an immediate and powerful action on the heart and caused complete arrest in less than an hour. The following analyses of the hearts and fluids showed that relatively small quantities of lactic acid were excreted into the perfusion fluid, but that it accumulated in the heart.

Time in min.	10	20	40
No. of exps.	8	4	12
Lactic acid excreted in mg. per g.	0·5	0·54	0·63

In ten hearts analysed after 40 min. anaerobiosis the lactic acid content was 1·66 mg. per g. Allowing for a control value of 0·66 mg. per g., this corresponds to an accumulation in 40 min. of 1·0 mg. per g. in the heart, and a total production of lactic acid of 1·63 mg. per g. in 40 min.

Table IV shows the outstanding effects produced by anaerobiosis in alkaline and in neutral Ringer's fluid. It would appear that in neutral Ringer's fluid the lactic acid produced cannot be excreted, and hence accumulates in the heart and produces paralysis fairly rapidly. The lactic acid content found after 40 min. perfusion is similar to the values shown in Table III for the lactic acid content of turtle's heart strips suspended in nitrogen and stimulated to exhaustion.

Table IV. Effect of anaerobiosis for 40 min.

	Alkaline Ringer's fluid (pH 8·5)	Unbuffered Ringer's fluid (pH 6·8–7·0)
Decrease in mechanical response as p.c. of normal	20	100
Lactic acid excreted into perfusion fluid mg. per g.	2·8	0·63
Lactic acid content of ventricle mg. per g. (control = 0·66)	0·75	1·66
Total lactic acid produced in mg. per g. in excess of control	2·9	1·63

In alkaline Ringer's fluid the lactic acid is excreted readily and the content inside the heart does not rise, hence the mechanical response is

but slightly impaired and the total production of lactic acid is considerably greater than it is in neutral Ringer's fluid.

Examination of the unbuffered perfusion fluid after arrest of the heart had occurred showed that its reaction was only very slightly acid (about *p*H 6·9). The frog's heart when supplied with oxygen can maintain a fair activity for long periods when perfused with a fluid with this reaction. The arrest of the heart that occurs in anaerobiosis with unbuffered Ringer's fluid must therefore be due to the accumulation of lactic acid in the heart itself rather than to any change in the reaction of the perfusion fluid caused by the acid that is excreted.

(vi) THE EFFECT OF GLUCOSE ON THE ASPHYXIATED HEART.

Freund and König [1927] found that hearts perfused with oxygen-free alkaline Ringer's fluid were arrested in 2 to 3 hours, but that when glucose was present the hearts maintained good contractions for at least 6 hours. They also showed that hearts arrested with oxygen lack could be revived by addition of glucose. Backmann [1927] found that hearts perfused with a solution containing sugar could survive anaerobically for as long as 61 hours. We confirmed Freund and König's conclusions, for we found that the addition of glucose enabled the heart to maintain a good activity for 6 hours, and that a heart arrested by lack of oxygen could be revived by glucose. The addition of as small a quantity as 2 mg. to 10 c.c. perfusion fluid produced an immediate stimulant effect on the exhausted anaerobic heart. The tracings published by Freund and König show that the stimulant effect produced by addition of sugar to the frog's heart exhausted by lack of oxygen is very dramatic as regards the rapidity and completeness of the recovery. Our results showed effects of similar intensity. This effect was only produced in alkaline Ringer's fluid, and the addition of glucose to a heart arrested by oxygen lack in neutral Ringer's solution produced no benefit. The figures given in Table V show that the anaerobic heart converts considerable quantities of glucose to lactic acid.

TABLE V. Effects of perfusion with oxygen lack for 6 hours with 0·1 p.c. glucose in the Ringer's solution.

No. of exps.	Average heart weight in g.	Average total carbohydrate p.c.	Lactic acid recovered from Ringer's fluid in mg. per g. per hour	Nitrogen excreted in mg. per g. per hour
6	0·076	1·59	5·7	0·02

The total carbohydrate content of the hearts after anaerobic perfusion with glucose for 6 hours, namely 1·59 p.c., was actually higher than that of the controls taken at the same time (average of 10 controls = 1·55 p.c. total carbohydrate). Therefore when the heart is perfused with an alkaline fluid containing glucose under anaerobic conditions it does not exhaust its own carbohydrate but obtains energy by converting the glucose present in the perfusion fluid to lactic acid. The nitrogen excretion is much lower in anaerobic than in aerobic conditions. Under aerobic conditions a heart perfused for 6 hours excreted 0·52 mg. nitrogen per g. [Clark, Gaddie and Stewart, 1931, Table III], which is about 25 times the value we obtained under anaerobic conditions. The trace of nitrogen obtained in the latter case cannot all be derived from the adenylic acid of the heart, if it be assumed that the pyrophosphate found by Clark, Eggleton and Eggleton [1931] represents the whole of the adenylic acid. It is noteworthy, however, that almost the whole of the nitrogen excreted by the anaerobic hearts was in the form of ammonia, whereas under aerobic conditions two-thirds of the nitrogen recovered was in the form of urea. It is of course possible that other substances than adenylic acid may undergo deamination without oxidation, especially under the abnormal condition of anaerobiosis.

(vii) Discussion.

Our results show that under anaerobic conditions the only important source of energy available for the isolated frog's heart is the conversion of carbohydrate to lactic acid. The heart, however, differs very widely from the skeletal muscle as regards the effect produced upon it by accumulation of lactic acid. The skeletal muscle can neutralize considerable amounts of lactic acid, whereas accumulation of lactic acid rapidly impairs the mechanical response of the heart and arrest occurs when the lactic acid content rises to 0·15 p.c. Consequently the heart is unable to function anaerobically, or to incur oxygen debt unless it is able to excrete rapidly the lactic acid it produces. The excretion of lactic acid depends on the reaction of the perfusion fluid. Excretion occurs freely at pH 8·5 and very slowly at pH 7·0. We have not yet investigated the intermediate range of reaction.

The effect of lack of oxygen on the frog's heart therefore depends primarily on the reaction of the perfusion fluid, for unless this is alkaline the heart is fairly rapidly arrested by lactic acid accumulating in its cells. If the perfusion fluid is alkaline the duration of activity depends on the amount of carbohydrate available. When the heart is perfused

with glucose-free alkaline Ringer's fluid its available carbohydrate begins to fail after about 2 hours, but if glucose is added the heart can maintain a good activity for many hours. Table VI shows the manner in which

TABLE VI. The duration of survival of the anaerobic frog's heart.

	Arrest from lack of oxygen min.	Mg. per g. lactic acid formed at time of arrest
Hearts poisoned with iodoacetic acid	<10	0
Hearts perfused, unbuffered Ringer's fluid	40	1·63
Hearts perfused, alkaline Ringer's fluid	120–180	5
Hearts perfused, alkaline Ringer's fluid + glucose	>400	>25

various modifications of the conditions of perfusion affect the duration of the survival of the anaerobic heart. It is interesting to note that a heart exhausted by perfusion for 3 hours with alkaline glucose-free Ringer's fluid still contains 0·77 p.c. total carbohydrate. This figure is considerably higher than that found in hearts after prolonged perfusion under aerobic conditions. For example, the authors in a previous paper [1931] recorded the following figures.

Method of perfusion	Total carbohydrate p.c.
Serum + Ringer's fluid 24 hours	0·480
Serum + glucose 24 hours	0·403
Serum + glucose + insulin 24 hours	0·280

The "availability" of the reducing substance in the frog's heart appears therefore to vary. About 0·3 or 0·4 p.c. cannot be utilized at all, whilst about 0·8 p.c. cannot be used under anaerobic conditions. About 0·15 p.c. reducing substance is non-fermentable [Clark, Gaddie and Stewart, 1932].

Under aerobic conditions the heart metabolizes both proteins and carbohydrates, whereas under anaerobic conditions it metabolizes carbohydrates alone. Our figures suggest that the protein metabolism in some manner makes available for metabolism a portion of the total carbohydrates.

In our previous work [Clark *et al.* 1931] we were puzzled by the fact that the isolated frog's heart, well supplied with oxygen, did not utilize glucose added to the perfusion fluid, whereas there was evidence that the isolated mammal's heart perfused with Ringer's fluid used considerable quantities of glucose, and other workers had reported glucose usage and stimulation by addition of glucose in the isolated frog's heart. These divergent results we believe to be due to variations in efficiency of oxygen supply, since any shortage of oxygen appears to stimulate the heart to break down carbohydrate.

The behaviour of the heart in regard to glucose in aerobic and anaerobic conditions forms a remarkable contrast. Under aerobic conditions the heart does not consume glucose added to the perfusion fluid and uses its own carbohydrate very slowly. Moreover, the addition of mono-iodo-acetic acid in concentrations sufficient to arrest all breakdown of carbohydrate to lactic acid does not produce any demonstrable effect on the activity of the heart as long as this is supplied with oxygen. Hence there is no direct evidence that the rapid breakdown of carbohydrate to lactic acid that occurs in anaerobiosis necessarily forms any part of the carbohydrate metabolism of the aerobic heart.

Conclusions.

1. The effect of oxygen lack on the heart depends on two variables, namely, alkalinity and sugar supply. The heart is rapidly arrested unless the perfusion fluid is alkaline. If the *p*H is 8·0 or over, the heart deprived of oxygen converts both its own carbohydrate or glucose added to the perfusion fluid into lactic acid, and thereby can maintain a good mechanical activity for a period of many hours.

2. In the presence of oxygen the heart uses much less carbohydrate, but even a slight oxygen deficiency can cause the production of some lactic acid.

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PHOSPHAGEN IN THE PERFUSED HEART OF THE FROG.

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The work presented in this communication was undertaken initially in order to ascertain whether phosphagen occupied the same position in the chemical changes accompanying contraction of cardiac muscle that it has been shown to hold in the case of skeletal muscle.

In the last few years a considerable body of fact has been accumulated concerning the function of phosphagen in the contraction of skeletal muscle of the frog. The view has been reached that the contractile process, which is non-oxidative in character even in the presence of oxygen, is energized by the breakdown of phosphagen, a process which can be reversed later by means of the energy accompanying the production of lactic acid (also an anaerobic process). This anaerobic restitution is not sufficiently complete to maintain the phosphagen content of the skeletal muscle at its normal level, but it appears greatly to retard the ultimate disappearance of the phosphagen. The small and variable heat evolution observed long ago by Hartree and Hill [1920] to occur during the minute or so following an anaerobic contraction (the "delayed anaerobic heat") appears to be the balance between the exothermic lactic acid formation and the endothermic restitution of part of the phosphagen broken down during the twitch [Lundsgaard, 1931].

The heart muscle of the frog contains normally only one-tenth of the amount of phosphagen held in reserve by a skeletal muscle, and it was natural though not logical to suppose that phosphagen was correspondingly less important in the former. A more probable interpretation of this difference is, however, that since the heart cannot be tetanized it need carry no more phosphagen than is required for the energy production of a single contraction. A heart of the size considered contains usually about 15γ of phosphagen phosphorus, a quantity which according to the calculation given later in this paper is probably twenty times greater than this minimal amount.

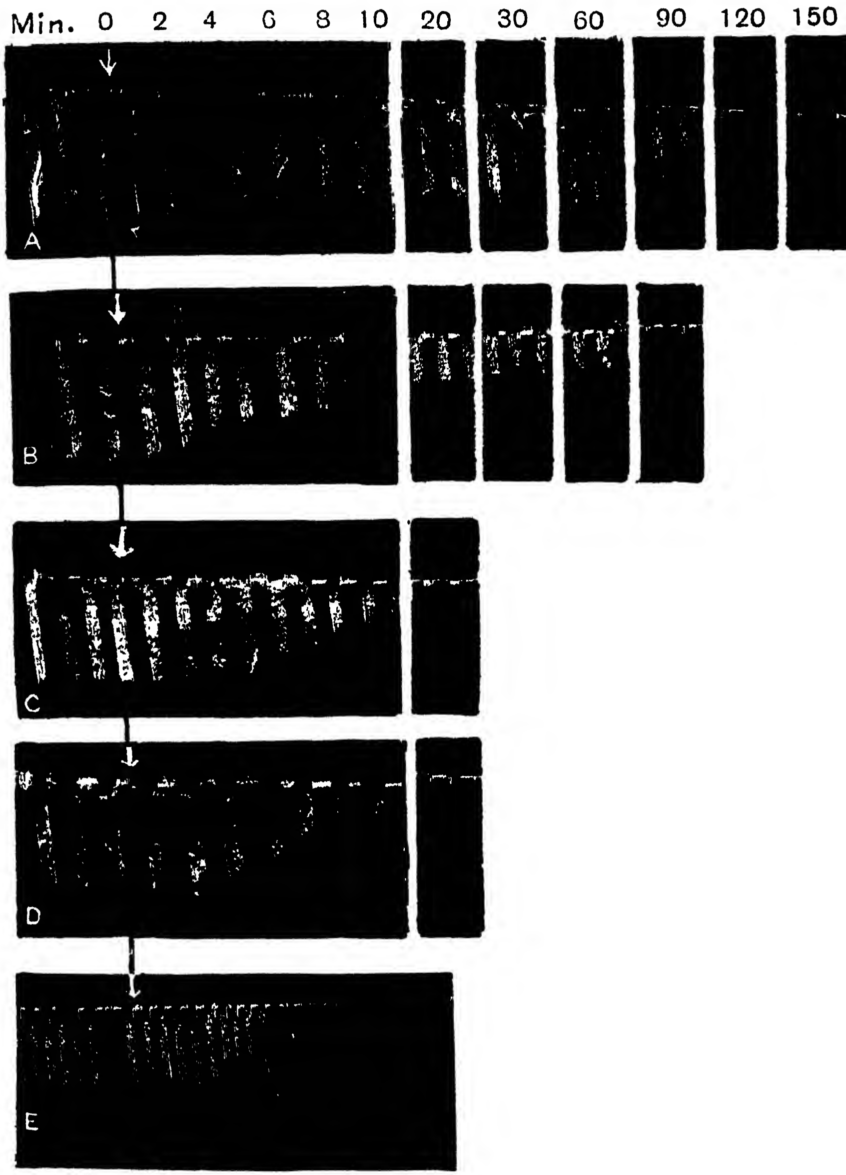

Fig. 1. Effect of anoxæmia on isolated frog ventricle perfused with various Ringer's fluids. Nitrogen commenced at zero time. A. Buffered alkaline fluid (pH 8·5). B. Buffered neutral fluid (pH 7·0). C. Buffered acid fluid (pH 6·5). D. Unbuffered fluid. E. Buffered alkaline fluid (pH 8·5) plus 1 in 50,000 monoiodoacetic acid.

A number of hearts from *R. esc.* (Hungary) were analysed by the method of Eggleton and Eggleton [1929], the acid-soluble phosphorus being measured in five fractions. To discover what gross changes resulted from a prolonged survival in Ringer's fluid other hearts were analysed after beating for 24 hours in 250 c.c. of oxygenated Ringer's fluid buffered with bicarbonate; the results have already been published [Clark, Eggleton and Eggleton, 1931]. The prolonged perfusion caused surprisingly small changes in the phosphate fractions, and in particular the phosphagen decreased only about 30 p.c.

The estimation of the five phosphate fractions requires a gram of tissue, a quantity which cannot be obtained from a single frog heart and requires often three or four. For the purposes of more detailed experiments therefore attention was confined to the orthophosphate and phosphagen of the heart, since these could be estimated with a fair degree of accuracy in as little as 100 mg. of tissue. Further, since for the purposes of this work spontaneous alterations of rhythm would constitute an unwanted variable, the preparation used for the subsequent experiments consisted of a ventricle mounted on a suitable cannula and excited at a speed of 14 beats per minute by induction shocks. The apparatus used has been described by one of us [Clark, 1927, Fig. 1]. It allowed free movement of fluid in the heart, except when records were taken when the conditions were approximately isochoric, and the extent of the excursions recorded in the tracings was nearly directly proportional to the pressure produced by the contraction.

When such a ventricle perfusing itself with neutral unbuffered Ringer's solution is deprived of oxygen, the strength of its beat begins to diminish within 3 min., and after 20 min. of oxygen lack the heart is very feeble (Fig. 1 D).

Allowing for the time taken by a stream of nitrogen to wash all the dissolved oxygen out of the perfusing fluid, the latent period of the tissue itself in such an experiment is probably less than 20 beats. In a number of such experiments the ventricle was cut down and analysed after periods of anaerobiosis varying from 3 to 30 min. The results (Table I) are depicted graphically in Fig. 2.

This is an effect analogous to the disappearance of phosphagen from a stimulated skeletal muscle, but phosphagen also disappears from resting skeletal muscle though only at one-hundredth of the pace. It was of interest therefore to discover whether phosphagen would have disappeared even from a quiescent heart, and if so whether the rate of disappearance would be less. Attempts to answer this question by means

of experiments on frog ventricle failed through the difficulty of obtaining it in quiescent condition. But it was found that the auricle of the

TABLE I. The effect of anaerobiosis on the frog's heart perfused with neutral and with alkaline Ringer's fluid.

No. of exps.	Ringer's fluid	Strength of beat	Time of anaerobiosis min.	A Phosphagen P mg. per 100 g.	B Inorganic P mg. per 100 g.	Phosphagen index (A/B)
5	—	Normal	0	7·2	11·5	0·63
3	Neutral	½–⅜ normal	5	2·0	12·4	0·16
3	,,	*ca.* ¼ normal	10	2·1	11·3	0·19
3	,,	Very feeble	15	0·9	10·1	0·09
1	,,	Nil	45	0·5	13·5	0·04
4	Alkaline	80 p.c. normal	5	2·9	11·7	0·25
2	,,	,,	10	2·7	10·2	0·27
5	,,	,,	20	1·7	13·5	0·13
1	,,	Weakening	70	2·5	14·5	0·17
1	,,	Very feeble	150	1·4	15·7	0·09

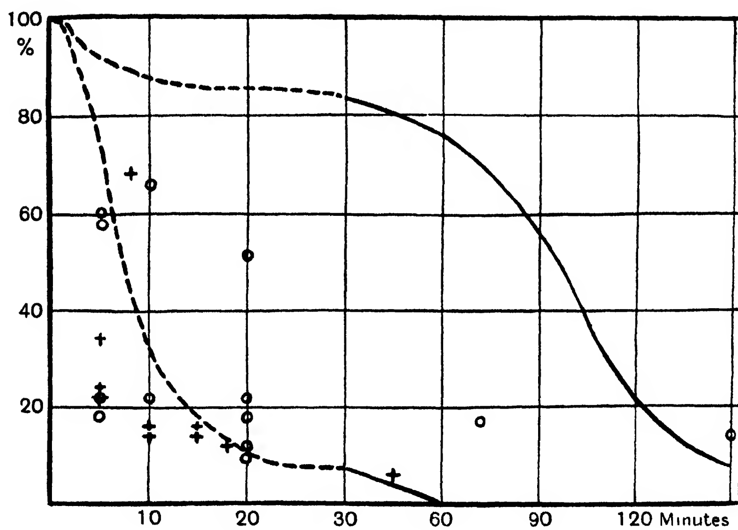

Fig. 2. Ordinate: p.c. of normal. Abscissa: time after exposure to nitrogen. Upper line: mechanical response with alkaline Ringer. Lower line: mechanical response with neutral Ringer. The crosses and circles show the phosphagen index expressed as the percentage of the normal value (Phosp. P./Inor. P. = 0·63 = normal phosphagen index). Circles—results with alkaline Ringer's fluid. Crosses—results with neutral Ringer's fluid.

tortoise heart presented no difficulty in this respect. Strips of this tissue, freed from sinus tissue, were suspended in Ringer's solution (buffered with bicarbonate), in which solution they showed no spontaneous activity.

The strips, sometimes stimulated, sometimes not, were deprived of oxygen and after an interval of 6 or 12 min. taken down and analysed (Table II).

TABLE II. The effect of anaerobiosis on the quiescent and active tortoise auricle.

No. of exps.	State of auricle	Time of anaerobiosis min.	Phosphagen P mg. per 100 g.	Inorganic P mg. per 100 g.	Phosphagen index
4	Aerated	0	9·4	9·5	1·0
1	Quiescent	12	2·5	9·1	0·27
1	,,	6	4·4	12·0	0·37
1	Beating	12	2·7	17·0	0·16
1	,,	6	1·14	13·2	0·085

The few figures obtained indicate quite clearly that the beating heart loses its phosphagen more rapidly than the quiescent heart, although the latter is extremely susceptible to lack of oxygen. In comparing the rates of disappearance of phosphagen in the resting and beating auricles with the much larger differences observed between resting and contracting skeletal muscle under anaerobic conditions it must be borne in mind that the basal metabolism of cardiac tissue amounts to one-quarter that of the maximum working metabolism, whereas in skeletal muscle this fraction is probably less than one-hundredth.

From Fig. 2 it can be observed that the rate of phosphagen disappearance is not noticeably altered if the Ringer's solution is buffered with bicarbonate, though the mechanical response of the ventricle to oxygen lack is profoundly different in this case. Martin [1905] showed that heart strips under anaerobic conditions were benefited by addition of alkali. Fig. 1, curves A, B and C, show the manner in which the reaction and amount of buffering of the perfusion fluid determine the effect produced by anaerobiosis. A heart arrested by oxygen lack in neutral Ringer's solution can be revived by addition of alkali.

At first sight the prolonged survival in alkaline Ringer's fluid of a ventricle deprived of oxygen and containing only a small amount of phosphagen would appear to contradict the hypothesis that phosphagen breakdown is the source of the energy of contraction and to favour the alternative view that the hydrolysis of phosphagen provides a buffering mechanism against acidity [Fiske and Subbarow, 1929]. The point was so important that parallel experiments were performed with the tortoise ventricle, an organ which, on account of its larger size, was expected to permit of more accurate analyses.

When set up in the same way as the frog's ventricle, in alkaline Ringer's solution (bicarbonate), a tortoise ventricle (weighing 1·5–2·5 g.)

beat regularly and well both under aerobic and anaerobic conditions. Unfortunately the chemical results were not as clear cut as one hoped owing to an unforeseen difficulty in the determinations (see Experimental Methods, section C). But it became clear that the ventricle would continue to beat well for long periods (up to 50 min.) in the absence of oxygen although it contained less than 1 mg. of phosphagen P per 100 g. of tissue (Table III).

TABLE III. The effect of anaerobiosis on the tortoise ventricle suspended in alkaline Ringer's fluid.

Time of anaerobiosis min.	Weight of ventricle g.	Phosphagen P mg. per 100 g.	Inorganic P mg. per 100 g.	Phosphagen index
0	1·8	<5	>5	$<1·0$
10	2·0	$>0·66$	9·1	$>0·07$
20	1·3	$>0·8$	15·6	$>0·05$
30	2·4	$>0·2$	16·1	$>0·01$
50	1·1	$<0·55$	8·6	$<0·06$

Both frog and tortoise ventricles are thus able to beat in the absence of oxygen when containing less than 1 mg. phosphagen P per 100 g. of tissue. Does this fact accord with the view that the hydrolysis of phosphagen is the sole source of energy for the contraction process? Since the phosphagen content remains unaltered at this level during relatively long periods of oxygen lack we must suppose that resynthesis of phosphagen in diastole keeps pace with its breakdown during systole. In other words the amount needed for a single beat must not be more than 1 mg. per 100 g.

Clark and White [1928], using a method similar to ours with *R. temp.*, found an oxygen consumption of about 1 c.c. per g. per hour when the ventricle filling was adequate. Clark, Gaddie and Stewart [1931], using the whole heart of *R. esc.* (Hungary) with natural rhythm (25–30 per min.), found an oxygen consumption of 1·3 c.c. per g. per hour in hearts perfused with Ringer's fluid.

In the case of a heart beating fifteen times a minute the oxygen usage is therefore less than 1·3 c.mm. per beat per g. tissue. The calorific value of oxygen is roughly 5 cal. per c.c. whatever foodstuff be burnt, and we may take the heat evolved per beat therefore as not more than $6·5 \times 10^{-3}$ cal. per beat per g. tissue. The resting metabolism of the heart is about one-quarter of the metabolism of a heart in moderate activity and therefore the heat production due to contraction and relaxation may be taken as 5×10^{-3} cal. per beat per g.

In skeletal muscle the heat of contraction is about equal to the heat of recovery, but from the work of Clark, Gaddie and Stewart [1932*b*]

on the production of lactic acid by the anaerobic frog's heart, it would appear that the recovery process in the heart is less efficient than it is in skeletal muscle. It is therefore safe to assume that less than half the total heat production occurs during the contraction process, and the latter may be taken as being not more than 2×10^{-3} cal. per beat per g.

The figure for the heat of hydrolysis of phosphagen given by Meyerhof [1930] is 11,000 cal.[1] per g. mol. (31 g. P), and on this basis 2×10^{-3} cal. are provided by the hydrolysis of $5{\cdot}6\gamma$ phosphagen P. This quantity per gram is equivalent to 0·56 mg. per 100 g. of heart tissue.

The figures given by Stella [1931] for the oxygen consumption of the tortoise ventricle lead to a somewhat lower value for that organ. Approximate though these figures are it nevertheless seems safe to conclude that the phosphagen breakdown per beat is normally of the order of 0·5 mg. per 100 g. of tissue, and might be expected to become rather less as the strength of the beat diminishes. It is therefore not impossible that the ventricles beating steadily in the absence of oxygen with phosphagen contents of just this order were obtaining their energy of contraction from this source and rebuilding the phosphagen molecules during diastole by making use of the energy released in the production of lactic acid.

The experiments of Fig. 1 (A, B and C) suggest strongly that the source of energy for the beating ventricle deprived of oxygen is the production, from some non-acidic precursor, of lactic acid. Whether this lactic acid production itself energizes the contraction or whether it provides the energy for resynthesis of phosphagen during diastole, the inhibition of this reaction might be expected to leave the ventricle with no motive power at all.

Iodoacetic acid is known to inhibit the production of lactic acid in skeletal muscles [Lundsgaard, 1930], and its effect on the frog's ventricle, in the presence and absence of oxygen, was naturally examined at this stage. This drug, in concentrations less than 1 : 5000, produced no apparent action in 2 hours on the ventricle adequately supplied with oxygen; but in anaerobic conditions it produced a rapid cessation of activity (Fig. 1 E).

A ventricle beating steadily with full oxygen supply shows no mechanical response to the addition of iodoacetic acid to its perfusion fluid in a concentration of 1 : 50,000, for periods as long as 2 hours, as shown

[1] This figure of 11,000 cal. was obtained by acid hydrolysis. Enzymic hydrolysis with muscle extracts gave a result of about 15,000 cal., which leads to an even smaller value for the phosphagen breakdown per beat (0·4 mg. per 100 g.).

in Fig. 3, nor is there any alteration in the ratio of phosphagen/inorganic phosphate in the tissue. If after 20 min. or so, when the drug has permeated the muscle, the air in the system is driven out by pure nitrogen, there is a very abrupt loss of mechanical power leading to complete arrest within 100 beats. There is a preliminary latent period of about 50 beats due, we believe, to the presence of oxygen in the system up to that time (Fig. 3). Since the perfusion fluid is buffered with bicarbonate, the ventricle would, in the absence of the drug, have gone on beating for at least an hour. A few minutes after the heart has ceased to beat it goes into a state of permanent and apparently irreversible contracture, and in this condition the phosphagen disappears completely.

TABLE IV. The effect of iodoacetic acid (IAA) on frog and tortoise ventricles.

Ventricle used	No. of exps.	Vol. of Ringer's fluid c.c.	Concentration of IAA	Atmosphere	Time min.	Strength of beat	Phosphagen P mg. per 100 g.	Inorganic P mg. per 100 g.	Phosphagen index
Frog	3	10	{1 : 12,500 {1 : 25,000	Air	40–100	Normal	4·9	8·2	0·6
,,	2	2	1 : 50,000	N_2	10– 20	Nil	1·8	11·0	0·17
Tortoise	2	10	1 : 20,000	H_2	12– 18	Very feeble	$<0·9$	9·6	$<0·095$
Frog	1	2	1 : 10,000	N_2	5	Very feeble	2·2	8·7	0·25
,,	1	2	1 : 5,000	N_2	5	Very feeble	1·9	11·2	0·17
,,	1	10	1 : 10,000	N_2	10	Steady contracture	0	12·0	0
,,	2	10	1 : 100,000	N_2	—	Nil	—	—	—
				Followed by O_2	—	Normal	6·0	13·4	0·45

More detailed consideration of these experiments raises a difficulty. The loss of mechanical function induced by iodoacetic acid is not accompanied by a parallel loss of the residual phosphagen from the heart. Only in the final stages, when the heart has practically ceased to beat, does this phosphagen begin to disappear, and the disappearance is not complete until a state of contracture has set in.

Admission of oxygen to the system before the state of contracture sets in results in a fairly complete functional recovery of the ventricle, and if analysed in this condition its phosphagen content is found to be approximately normal (Table IV). Indeed, by alternate administration of air and nitrogen, the ventricle can be reversibly arrested several times in succession (Fig. 3).

It is obvious that the drug is not a mere general protoplasmic poison, but is exerting a highly specific action on some process or structure of the heart which is only required during anaerobic activity. No certain

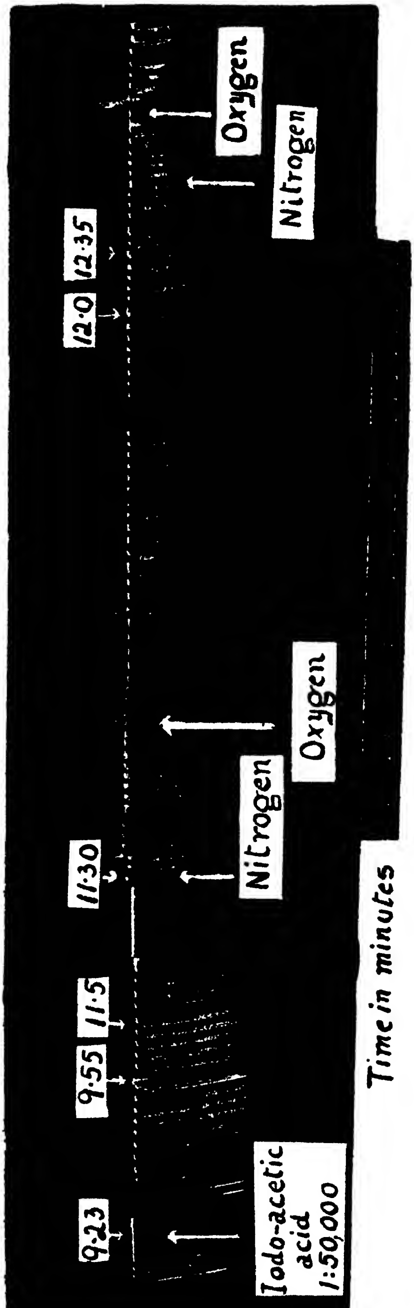

Fig. 3. Effect of iodoacetic acid on frog ventricle perfused with alkaline Ringer's fluid under conditions alternately aerobic and anaerobic.

effect was produced by 10γ of iodoacetic acid in 2 c.c. of perfusion fluid, whereas 20γ sufficed to arrest completely a ventricle of average weight; but a dose of this magnitude may take as much as 20 min. to arrest the ventricle, whereas a dose ten times greater will bring about the arrest in a few seconds. It is clear therefore that the poisoning action can be graded.

Since it has been shown that iodoacetic acid inhibits the production of lactic acid in the heart as it does in skeletal muscle, it follows that, although there is no alternative to this source of energy in the anaerobic heart, there must be alternative sources for the aerobic heart. Evidence for the existence of such alternative sources has been found by Clark and his colleagues [1932 *a*] who showed that 60 p.c. or more of the energy of the normal aerobic heart was derived from the combustion of substances other than carbohydrate.

We made a few experiments to see whether depression or arrest of the heart by various agencies was necessarily accompanied by reduction of phosphagen. Any effect that interferes with the oxygen supply of the heart will cause phosphagen loss, and we endeavoured to prevent this effect by emptying and filling the hearts rhythmically by changes of external pressure when they were arrested. The results shown in Table V indicate that a considerable variety of depressant agents can depress or arrest the heart without causing a proportionate reduction in its phosphagen content.

TABLE V. The effect of various depressant agents on the frog heart.

Depressant agent	Time of action min.	Strength of beat	Weight of heart g.	Phosphagen P mg. per 100 g.	Inorganic P mg. per 100 g.	Phosphagen index
Calcium lack	35	Nil	0·2	5·4	6·8	0·8
Potassium excess	30	50 p.c. (irreg.)	0·3	6·0	5·0	1·2
Potassium excess	10	Nil	0·22	4·6	10·5	0·44
Ethyl urethane 0·2 *M*	20	Nil	0·35	4·8	10·9	0·44
Ethyl urethane 0·15 *M*	15	Nil	0·23	4·4	12·2	0·36
Acetyl choline	25	40 p.c.	0·26	4·7	10·3	0·45
Acetyl choline	10	40 p.c.	0·37	4·5	9·6	0·47

One generalization seems to emerge from this work. If a heart is beating or is in such a condition that it can be made to beat by a suitable alteration in its surroundings (*i.e.* electrical stimulation, administration of oxygen, addition of calcium, etc.), it is always found to contain phosphagen. The amount may be in some circumstances smaller than normal, but not smaller than would be needed to supply the energy for a single contraction.

Experimental methods.

A. The nitrogen used in all experiments was purified by passing through a quartz tube filled with copper filings for 18 in. of its length. The middle 6 in. of the copper filings were heated to dull red heat by a gas flame. This is too high a temperature for copper to combine with oxygen, but there are necessarily two zones, one on each side of the heated length, which are at the right temperature for this reaction. The first zone met by the incoming gas invariably darkened in the course of an experiment. The absence of any darkening in the neighbourhood of the second zone gave assurance that the removal of oxygen was as complete as could be effected by copper. The surface of the copper was renovated occasionally by passing hydrogen through it whilst the tube was being heated.

B. The iodoacetic acid was prepared by the interaction of chloracetic acid and potassium iodide in strong aqueous solution. The acid was freed from iodine by repeated solution in benzene and reprecipitation with petroleum ether in which it is comparatively insoluble.

C. The estimation of phosphates in a single frog ventricle presents certain difficulties not met in skeletal muscle. The first is the impossibility of assessing the weight of the tissue with any accuracy, particularly if the weighing must be done before the experiment begins. The practice was adopted of weighing the intact heart together with any fluid it might contain. Some preliminary experiments indicated that the weight so measured was approximately double the weight of the ventricle after the latter had been cut open and squeezed gently between layers of filter paper. But worse than this constant error was the very large variable error. We are of the opinion that it is impossible to estimate the weight of a ventricle before its use in an experiment to within less than ± 15 p.c. For the purposes of the present study this difficulty was minimized by expressing the condition of a ventricle in terms of the ratio phosphagen P/inorganic P, for since the sum of the two has been found to remain approximately constant so long as the heart is alive, their ratio provides a more accurate as well as a more sensitive criterion of the physiological condition of the muscle.

The second difficulty arises out of the smallness of the quantities of phosphorus to be measured. The average ventricle in the foregoing experiments contains about 30γ of phosphagen P + orthophosphate P. Of this, phosphagen P sometimes amounted to less than 2γ. A quantity of the order of 2γ can be estimated by the Briggs' colorimetric tech-

nique, but not with any claim to accuracy: it is our experience that one can estimate to the nearest 1γ.

The technique adopted was that published in this *Journal* by Eggleton and Eggleton [1929], with certain modifications. The muscle was ground in a mortar with 10 c.c. 4 p.c. trichloroacetic acid, filtered immediately, and the filtrate was measured and at once neutralized with baryta. It was found an advantage to use baryta contaminated with barium carbonate. The greater bulk of precipitate obtained facilitated the separation of the insoluble orthophosphate from the soluble creatinephosphate. The heart muscle not only contains less acid-soluble phosphorus than skeletal muscle, but it contains some material, perhaps of lipoid nature, which tends to hinder the clean precipitation of barium phosphate. Tortoise ventricles were particularly prone to this behaviour, and it was found necessary to add an excess of baryta and pass in carbon dioxide in order to obtain clean filtrates. Whilst this treatment ensured the absence of inorganic phosphate from the phosphagen fraction, it also caused the removal of some phosphagen, possibly adsorbed on the barium carbonate. The results given in Table III are, with one exception, low on this account.

The absence of inorganic phosphate from the soluble (phosphagen) fraction was tested by analyses of hearts dead for 24 hours. In no case was any phosphagen detected.

Summary.

1. Phosphagen disappears from a tortoise auricle strip deprived of oxygen faster if the strip is contracting rhythmically than if it is quiescent.

2. The phosphagen index (the ratio phosphagen P/orthophosphate P) of the tortoise ventricle falls in the absence of oxygen from a value of about 1 to a value of about 0·06 in 10 min. or so. It remains at about this value for at least 45 min.

3. The frog ventricle shows similar behaviour; the index falls from about 0·6 to 0·1 or less. This is accompanied by a parallel loss of mechanical activity if the perfusion fluid is neutral and unbuffered, but if the latter is buffered with bicarbonate the loss of mechanical activity is greatly delayed. It is still 80 p.c. of the normal after an hour's anaerobiosis.

4. No effect is produced either on the phosphagen index or upon the mechanical response by iodoacetic acid in concentrations up to

1: 5000 in the perfusion fluid, provided this fluid is aerated. If all traces of oxygen are suddenly removed, activity ceases in the course of 10–100 beats, according to the concentration of the drug. This action is reversible by oxygen within the limits described. 20γ of iodoacetic acid in 2 c.c. of perfusion fluid will suffice to arrest a ventricle weighing 0·25 g.

5. Calcium lack in the perfusion fluid arrests the ventricle even more rapidly than oxygen lack, but leaves the phosphagen index unaltered, and various other depressant agents exert a similar action in a less marked degree.

6. It is suggested that in all circumstances the hydrolysis of phosphagen is the immediate source of the energy of contraction of the heart: that, in the presence of oxygen, protein and carbohydrate are burned to provide energy for the resynthesis of phosphagen during diastole; and that, in the absence of oxygen, lactic acid production is the only reaction available for this purpose.

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THE INTERDEPENDENCE OF GASTRIC SECRETION AND THE CO_2 CONTENT OF THE BLOOD.

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For some years sporadic indications have appeared in the literature that there is a relation between blood CO_2 content and gastric secretion. Maly's theory, first advanced in 1878, postulated the importance of CO_2 in the formation of hydrochloric acid, only, however, secondarily to NaH_2PO_4 which he believed to be the compound directly concerned in the formation of HCl. F. Delhougne [1927] found a decreased acidity in response to the alcohol test meal when the subjects hyperventilated. He found, however, an increase in the CO_2 combining power of the blood under these conditions, which makes the effectiveness of the hyperventilation doubtful. Bakaltschuk [1928] demonstrated an increase in gastric acidity in human subjects after the inhalation of CO_2. An increase was also obtained by Apperley and Semmens [1928] after the rebreathing of the subject's own expired air. In a different type of experiment Szilard [1930] showed an augmented "fasting" secretion following intravenous injection of $NaHCO_3$; the injection of glucose on the other hand caused no such rise. Numerous authors have observed that feeding large quantities of $NaHCO_3$ over prolonged periods of time raises the level of the acid curve during fractional test meals [Linoissier and Lemoine, 1894; Crohn, 1918]. The ingestion of sodium bicarbonate by patients on a Sippy diet in a large number of cases increases the CO_2 content of the blood [Hardt and Rivers, 1923; Koehler, 1927; Gatewood and others, 1928]. Working on dogs, Boyd [1924] observed that, in small doses, sodium bicarbonate tends to increase gastric secretion. Apperly and Semmens [1928] showed, in human subjects, that there was a parallelism between blood bicarbonate content and the response to a standard test meal. They attributed this to a common effect of variation in oxygenation of the blood on both gastric secretion and blood bicarbonate content.

The results reported by the above investigators have been obtained upon human subjects using the gastric test meal. This criterion of gastric secretion is open to serious objection, since the values obtained depend not only on the activity of gastric cells, but also on the emptying time of the stomach, and regurgitation of duodenal contents. Dilution due to salivation and to the ingested meal, and conditioned reflex effects are also sources of error.

Apperly and Crabtree [1931] in a very recent article are cognizant of the above sources of error (as indeed were Apperly and Semmens), and believe that they have taken them into account. In this article, they report a direct relation between the concentration of gastric hydrochloric acid and the plasma bicarbonate content.

In 1929, during an experimental investigation of the production of gastric secretion by vagal stimulation in dogs [Vineberg, 1931], it was observed that secretion was not obtained following excessive artificial respiration, but commenced shortly after the cessation of hyperventilation. The experiments reported in this paper were undertaken in an attempt to elucidate these results. In a preliminary report of this investigation [Vineberg and Browne, 1931] the conclusion at which we arrived was that a definite interdependence existed between the CO_2 content of arterial blood and the character of gastric secretion.

Methods.

Dogs ranging in weight from 8 to 25 kg. were employed. All animals were fed meat and porridge for 3 days, and milk only for the 24 hours immediately preceding the operation. Anæsthesia was induced by means of ether and continued by intravenous injection of a chloralose-urethane mixture (1 g. chloralose, 10 g. urethane in 60 c.c. normal saline: dose, 3 c.c. per kg.). A tracheal tube was inserted, both carotid arteries were isolated and tied, the vagus nerves were carefully separated from the carotid sheath and severed. The œsophagus was tied at the level of the first tracheal ring in order to prevent contamination of gastric juice with saliva. A metal fistula was inserted into the anterior wall of the stomach and firmly secured. The stomach was isolated from the duodenum by means of a ligature secured around the pylorus, thus preventing regurgitation. The anterior abdominal wall was closed around the metal fistula and the animal placed on a stand in the prone position. In one group of experiments, gastric secretion was obtained by electrical stimulation of the vagus nerves; in another group, subcutaneous injection of histamine (ergamine acid phosphate) was the gastric stimulant employed.

Secretion was collected in graduated centrifuge tubes at definite intervals. The volume of each sample was measured, the free and total acidity were determined by titration, using Töpfer's reagent and phenolphthalein as indicators. The total chlorides were determined by the method of Wilson and Ball [1928]. Blood was withdrawn from one carotid artery, the other being used for recording the blood-pressure. All blood samples removed from the carotid were collected and centrifuged under paraffin oil. The time of centrifuging, using the Angle centrifuge, was usually under 2 min., so that loss of CO_2 was minimized. The CO_2 content was determined on the true plasma, without equilibration, by the method of van Slyke [1917]; plasma *p*H was estimated by Cullen's colorimetric procedure [1922]; the chlorides in blood and plasma by the method mentioned above. Lactic acid in whole blood was determined by the method of Friedemann, Cotonio and Shaffer [1927] (expressed as mg./100 c.c. of whole blood).

Experiments.

The experiments have been divided into the following groups, and those described are illustrative of other similar experiments.

(1) *Nerve stimulation:*

Influence of: A. Hyperventilation;
B. Injection of acid (HCl and lactic);
C. Injection of NaCN.

(2) *Histamine stimulation:*

Influence of: A. Hyperventilation;
B. Injection of acid (HCl and lactic, and of base $NaHCO_3$);
C. Injection of NaCN.

(1) *Nerve stimulation.*

A. *Hyperventilation.*

We present two examples of this type.

In this experiment (Fig. 1) the vagus nerves were stimulated with an induction current interrupted 15 times per minute, using the left and right vagi alternately for periods of 10 min. each. Collections of gastric juice were made in periods of 10 min.; these represent corresponding periods of nerve stimulation. Samples were taken in this way in order to follow variations in nerve response. The difference between

right and left nerves is seen in the figures and is especially marked in the volume output. It will be observed that after 120 min. of stimulation, a satisfactory flow of gastric secretion was obtained and showed the values for free and total acidity usual with this type of stimulation. During this period the arterial plasma CO_2 content gradually rose to a

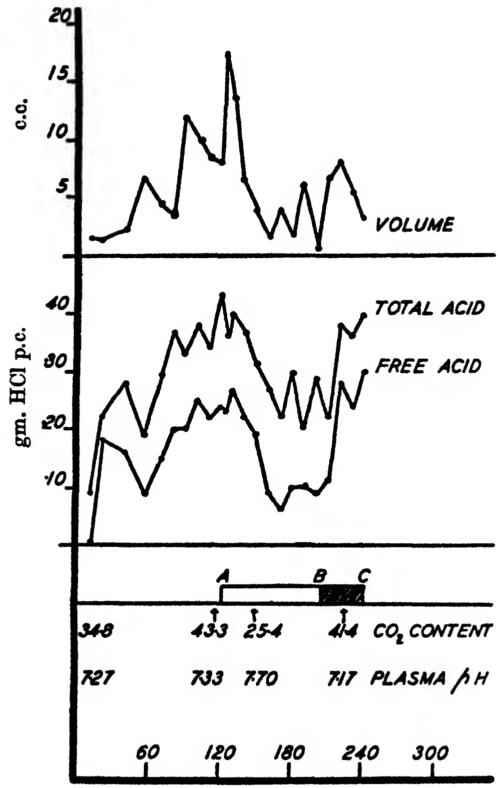

Fig. 1. Dog 9. Wt. 13·3 kg. Vagal stimulation. *A–B*, artificial ventilation at the rate of 64 per min. with air. *B–C*, the same rate of ventilation. 8 p.c. CO_2 in air.

value of 43·3 vol. p.c. (this figure is approximately normal for the dog under these conditions). At the point *A* artificial hyperventilation was applied at the rate of 64 per min. for a period of 80 min. The pump used had a stroke volume of 1700 c.c. There was a rapid fall in the volume output; 20 min. later the free and total acidity also fell. Coincidental with these changes the plasma CO_2 content fell to 25·4 vol. p.c. In other experiments we have found that the CO_2 content usually reaches an approximately constant low level within 20 min. after the application

of hyperventilation. The diminished acidity continued for 60 min. At this stage (*B*) a Douglas bag containing a mixture of 8 p.c. CO_2 and air was connected to the pump inlet. The rate of hyperventilation was kept constant. The acidity rose immediately to control levels; the CO_2 content

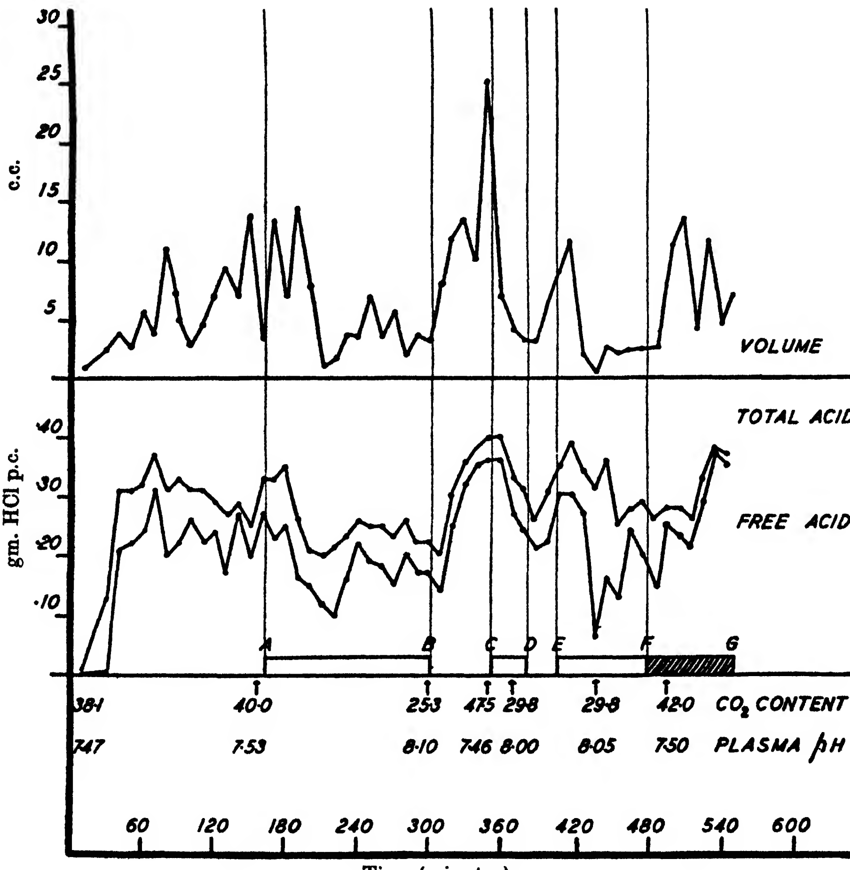

Fig. 2. Dog 10. Wt. 16 kg. Vagal stimulation. *A–B*, artificial ventilation at the rate of 74 per min. with air. *C–D* and *E–F*, the same. *F–G*, the same rate of ventilation. 5 p.c. CO_2 in oxygen.

of the plasma also rose reaching a level of 41·1 vol. p.c. in 27 min.; the *p*H fell below control levels.

A second experiment is presented in Fig. 2. Secretion was obtained following vagal stimulation and maintained for a period of 50 min. Hyperventilation applied at *A* at the rate of 74 per min. reduced the volume of secretion, free and total acidity and plasma CO_2 content.

Secretion practically ceased for a period of 100 min. Hyperventilation was discontinued at *B*, and the animal resumed a normal respiratory rhythm. The volume, free and total acidity rose to and above control values, as did the CO_2 content. This procedure was repeated for a shorter period with similar effects (as may be seen in the figure *C* to *D*). A third application of hyperventilation (*E* to *F*) again reduced all values, and these were restored following the introduction of 5 p.c. CO_2 at *F*. In neither hyperventilation experiment was there any significant change in the total chloride content of the gastric secretion.

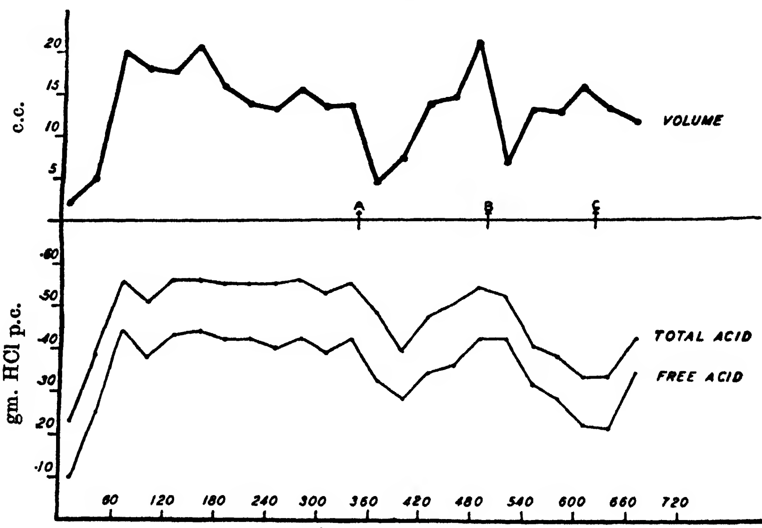

Fig. 3. Dog 36. Wt. 15 kg. Vagal stimulation. *A*, *B* and *C*, vagus ceased acting on heart. Position of electrodes changed.

In a control experiment presented in Fig. 3, stimulation of the vagus produced gastric secretion which gradually rose to reach its height in 1 hour. For a period of 5 hours secretion was maintained at a constant level of volume and acidity. This is longer than the total duration of the hyperventilation experiments. At the end of the 5 hours (point *A* in the figure) the volume of secretion diminished as did the free and total acidity.

This decrease was due to a diminished excitability of the nerve at the point of contact with the electrodes. The values were returned to previous levels by moving the electrode to a fresh position on the nerves. This procedure was repeated on two subsequent occasions. At the end of the experiment the nerves were acting and the dog was in good con-

dition. The total period of stimulation was over 10 hours. We have considered the vagus nerve as acting satisfactorily when a typical effect on heart rate and blood-pressure resulted at each stimulation. In the experiments shown in Figs. 1 and 2 and in Table I, the nerves acted satisfactorily throughout according to this criterion.

TABLE I. Dog 8. Wt. 17·4kg.: nerve stimulation.

Sample No.	Time min.	Volume c.c.	Free acidity g. p.c.	Total acidity g. p.c.	CO_2 content vol. p.c.	*p*H
0	Contents	12·8	0·00	0·10	—	—
1	15	1·7	0·00	0·07	—	—
		Stimulation current: 1 millivolt				
2	20	1·3	0·00	0·10	—	—
3	20	1·5	0·06	0·16	—	—
4	20	4·4	0·09	0·20	—	—
5	20	6·2	0·15	0·23	—	—
6	20	—	0·22	0·31	—	—
7	10	5·0	0·29	0·37	—	—
8	10	4·4	0·25	0·36	—	—
9	10	6·5	0·34	0·40	—	—
10	10	6·0	0·25	0·37	43·3	7·3
11	10	6·7	0·32	0·44	—	—
12	10	5·5	0·26	0·36	—	—
13	10	5·6	0·28	0·40	—	—
14	10	5·5	0·30	0·37	—	—
		Acid injection commenced				
15	10	5·4	0·33	0·42	—	—
		Total of 75 c.c. of 0·5*N* HCl injected				
16	10	4·6	0·35	0·47	43·3	7·0
17	10	3·7	0·33	0·45	—	—
18	10	0·5	0·25	0·43	24·2	7·1
19	10	0·3	0·15	0·33	—	—
20	30	0·2	—	—	—	—

B. *Acid injection.*

In this experiment (Table I) over a period of 80 min. an average rate of secretion of 5·6 c.c. for 10 min. was maintained. The average for the free acidity was 0·29 g. HCl p.c. and for the total acid 0·38 g. HCl p.c. The CO_2 content was 43·3 vol. p.c. and the *p*H 7·33. In a period of 9 min. 75 c.c. of 0·5*N* HCl were injected intravenously; immediately after the injection the plasma CO_2 content was still 43·3, but the plasma *p*H had fallen to 7·00. The respiration of the animal had not yet increased. The secretion continued throughout the injection. The volume gradually fell over the 20 min. period following the cessation of acid administration. In contrast to this the free and total acidity showed a slight rise. When this length of time had elapsed, the secretion suddenly stopped and did not return for the remainder of the experiment. A blood

sample taken 10 min. after the cessation of secretion showed 24·2 vol. p.c. of CO_2 and a pH of 7·10.

The dependence of secretion upon CO_2 content rather than upon blood pH is indicated in the above experiment.

C. *Injection of* NaCN.

Since it has been shown that hyperventilation by changing blood pH has the effect of shifting the dissociation curve of hæmoglobin in such a way that it yields oxygen less readily to the tissues, the possibility that the effect of hyperventilation may be due to interference with the oxygen supply of the gastric mucosa must be considered. It was decided to inject sodium cyanide as a means of interfering with tissue oxidation. Repeated injections of 7 c.c. of $N/100$ NaCN were given intravenously. The total amount given was 41 c.c. in 1 hour. Despite this the gastric secretion was not affected. Although the lethal dose for a 14 kg. dog was 30 c.c. given in a single injection, we do not know the degree of interference with tissue oxidation produced in the above experiment. The changes in the dissociation curve of hæmoglobin are dependent upon those in pH. The significance of the latter will be discussed later.

After the above results were obtained, the possibility of an altered plasma CO_2 and pH affecting the vagus nerve endings was considered. This was rendered improbable in view of the continued action of the vagus on the heart during hyperventilation. In an endeavour to exclude this possibility, histamine was used as it is considered to act directly upon the parietal cells [Popielski, 1920; Vineberg and Babkin, 1931]. The operative procedure was identical with that of nerve experiments. The vagi were severed but not stimulated.

(2) *Histamine stimulation.*

A. *Hyperventilation.*

In Fig. 4 it is seen that after a 20 min. period of secretion, artificial respiration was applied and continued for 60 min. (*A–B*) without any effect upon the course of the secretory activity. It was then discontinued for 50 min. without any effect. The dog was again hyperventilated for 100 min. (*C–D*), the volume and acidity remaining practically constant although the plasma CO_2 was lowered to 24·7 vol. p.c. and pH raised to 7·80. 5 p.c. CO_2 was introduced (*D–E*) with the rate of hyperventilation kept constant for a period of 30 min. This raised the CO_2 content of the plasma to 61·0 and lowered the pH to 7·03. The effect of the CO_2 was to diminish the secretion both in volume and acidity. This effect persisted for 45 min. after the CO_2 had been discontinued; hyperventi-

lation was maintained throughout during the following 70 min. (*E–F*). The volume gradually rose; the free and total acidity also rose, but the former did not reach its previous value.

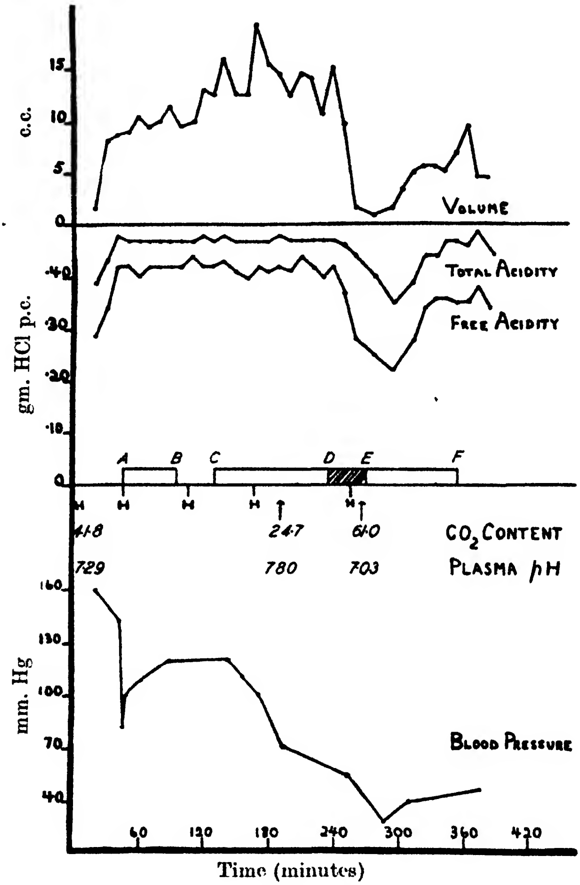

Fig. 4. Dog 12. Wt. 14·3 kg. At *H*, 4 mg. histamine subcutaneously. *A–B*, artificial ventilation at the rate of 76 per min. with air. *C–D*, the same. *D–E*, the same rate of hyperventilation. 5 p.c. CO_2 in oxygen. *E–F*, the same rate with air only.

A second experiment is shown in Fig. 5. In this experiment CO_2 and hyperventilation were applied (*A–B*) as soon as secretion had been established. The volume decreased gradually from the first, but the free acidity rose slightly and the total acidity remained constant for 50 min. and then both fell off abruptly. The CO_2 content of the plasma rose only slightly above control levels. The administration of CO_2 was

then discontinued and hyperventilation was continued at the same rate as before (*B–C*). The volume gradually increased, the free and total acid however remained low. Then 8 p.c. CO_2 was again used in the inspired air (*C–D*). The recommencement of CO_2 was followed by a

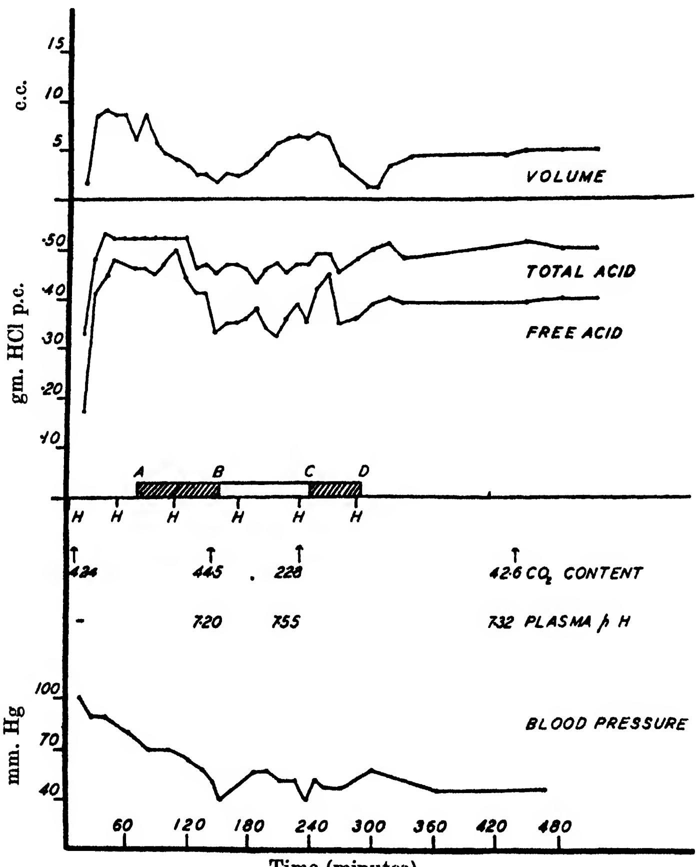

Fig. 5. Dog 14. At *H*, 4 mg. histamine subcutaneously. *A–B*, artificial ventilation at the rate of 74 per min. with 5 p.c. CO_2 in oxygen. *B–C*, same rate of ventilation with air only. *C–D*, with 5 p.c. CO_2 in oxygen.

20 min. period in which a definite rise in free acidity took place. There was no change in volume. This was followed by a decrease in both volume and acidity continuing for 30 min. At this point (*D*) the dog was permitted to resume a normal respiratory rhythm, breathing air only. All values immediately rose and were maintained at an approximately constant level for 3 hours with no further injections of histamine.

Histamine is an extremely powerful gastric stimulant, and it is seen from the above results that once secretion is well established under its influence, hyperventilation has no effect. However, from Fig. 5 there are indications that once histamine secretion has been depressed the hyperventilation tends to keep the values reduced.

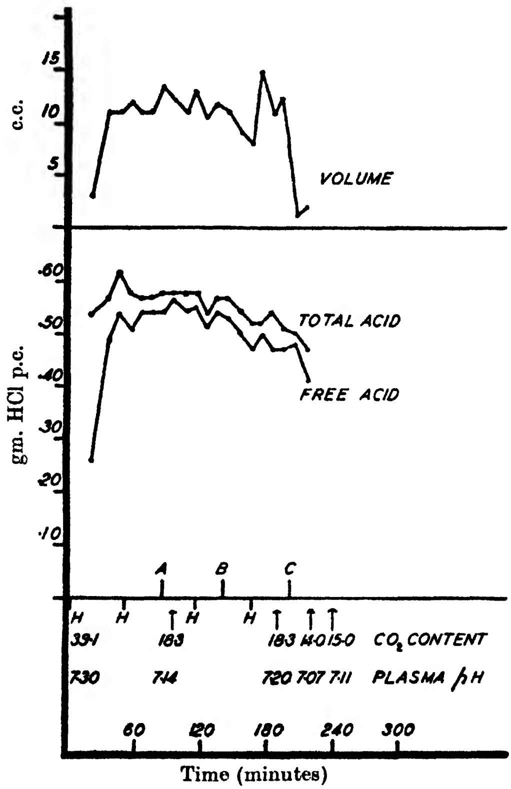

Fig. 6. Dog 16. Wt. 10·3 kg. At *H*, 4 mg. histamine subcutaneously. At *A*, 40 c.c. 0·5*N* HCl intravenously in 5 min. At *B*, 40 c.c. 0·5*N* HCl intravenously in 3 min. At *C*, 23 c.c. 0·5*N* HCl intravenously in 6 min.

B. *Injection of acid.*

In the experiment illustrated in Fig. 6 secretion was maintained by histamine at a constant level for 70 min. Half normal hydrochloric acid was injected intravenously (*A*) (40 c.c. in 5 min.). For the next 40 min. no change in the character of secretion took place. A further 40 c.c. of acid was then injected (*B*) in 3 min. The values decreased only slightly for the next 60 min. A third injection (*C*), 23 c.c. in 6 min., was made.

Secretion stopped abruptly and 55 min. later the dog died. The blood CO_2 content was at the usual level at the beginning of the experiment, and, following the first injection of acid, fell and declined with each subsequent injection of acid. The *p*H also decreased over these periods.

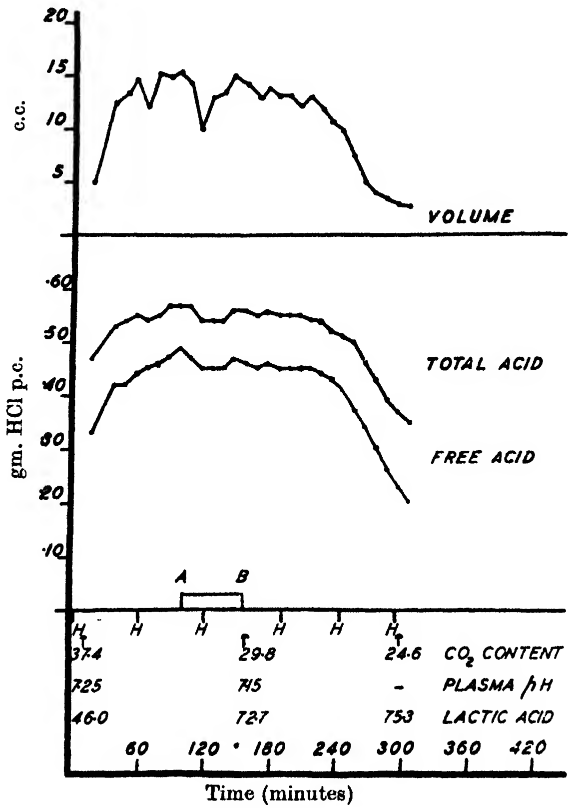

Fig. 7. Dog 21. Wt. 23·3 kg. At *H*, 4 mg. histamine subcutaneously. *A–B*, 120 c.c. 0·5*N* lactic acid intravenously at the rate of 3 c.c. per min.

Because HCl is the acid of gastric secretion, and because it does not enter the blood in physiological conditions, it was decided to use lactic acid which occurs in normal metabolism. Fig. 7 demonstrates the absence of effect of the injection of large quantities of lactic acid on histamine secretion after it has been established. The CO_2 content and *p*H of the plasma were lowered and the lactic acid content of the blood rose. Toward the end of the experiment secretion gradually declined. The death of this animal was due to an overdose of anæsthetic.

From Figs. 6 and 7 it may be seen that under conditions of low plasma CO_2 content and low pH, histamine secretion, unlike that

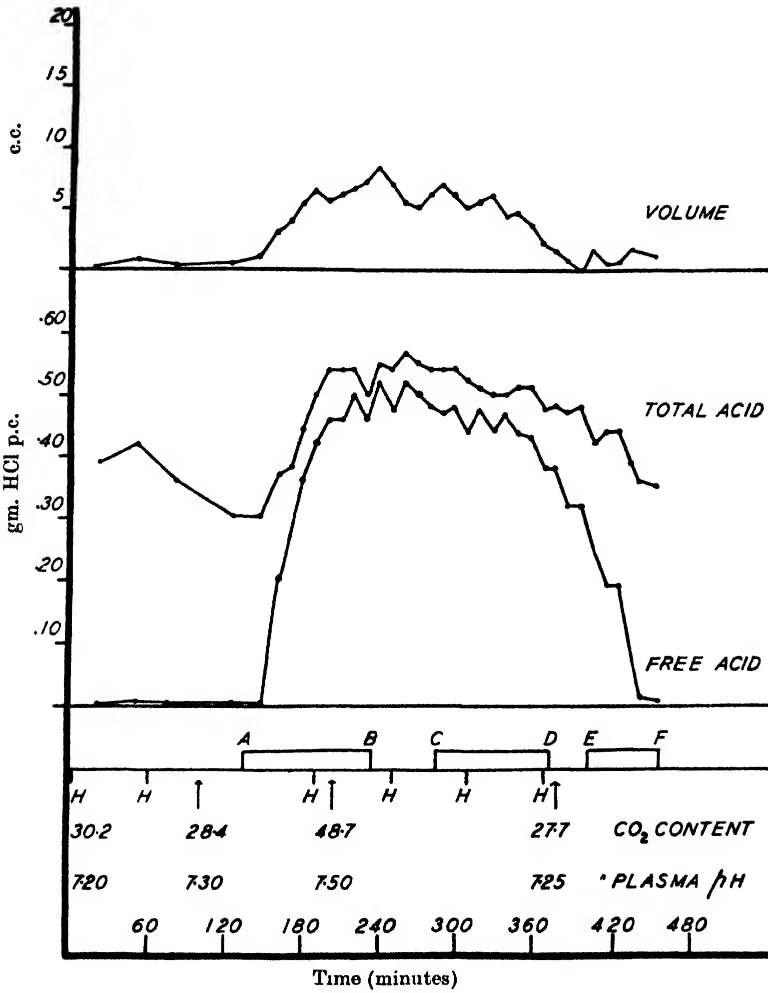

Fig. 8. Dog 17. Wt. 14·5 kg. At *H*, 4 mg. histamine subcutaneously. *A–B*, 47 c.c. 10 p.c. sodium bicarbonate intravenously at the rate of 0·5 c.c. per min. *C–D*, 120 c.c. 0·5 *N* HCl intravenously at the rate of 1·3 c.c. per min. *E–F*, 75 c.c. 10 p.c. sodium bicarbonate intravenously.

produced by vagal stimulation, continues for long periods unaffected. If, however, these conditions of low CO_2 content and low pH are in existence before the histamine is injected, it fails to produce a flow of

gastric juice. This occurred in three experiments. The acidosis occurred spontaneously for unknown reasons. One of these experiments is illustrated in Fig. 8. At the commencement of the experiment the plasma CO_2 was 28·4 vol. p.c. and pH 7·30. Repeated doses of 4 mg. of histamine were given at hourly intervals. The usual latent period following this dose of histamine is not more than 20 min. In this experiment there was no secretion for 145 min., but it appeared 15 min. after the commencement (*A*) of an intravenous injection of 10 p.c. sodium bicarbonate and was well maintained for a period of 120 min. Hydrochloric acid was then injected (*C–D*), and in this case the secretion gradually diminished and finally ceased. When secretion was well established, the CO_2 content was 48·7 vol. p.c. and the pH 7·5. Following the injection of acid when secretion ceased the CO_2 content was 27·7 and the pH 7·27. It will be observed that a period of 85 min. elapsed between the last histamine injection and the commencement of secretion after the injection of $NaHCO_3$ intravenously. This indicates that the inhibition of gastric secretion under conditions of initial acidosis is not due to a rapid destruction of the histamine injected.

A similar inhibition of secretion under conditions of initial acidosis occurred in vagal stimulation experiments, and as in the case of histamine a flow of gastric juice was established following the injection of sodium bicarbonate. In another case of initial acidosis where no injection of sodium bicarbonate was given, acidosis progressively increased and repeated hourly injections of histamine failed to stimulate the gastric glands. The animal died 5 hours after the first histamine injection. The injection of 250 c.c. of normal saline failed to induce secretion. This indicates that dehydration is not the cause of the inhibition of secretion.

C. *Injection of sodium cyanide.*

As in the case of nerve stimulation, repeated doses of 7 c.c. of $N/100$ sodium cyanide at 10 min. intervals over a long period of time had absolutely no effect on the functioning of the gastric mucosa. The characteristic effect of sodium cyanide on respiration and blood-pressure was obtained at each injection.

In all the histamine experiments the standard dose used was 4 mg. injected every hour, and in numerous experiments we have found that once a satisfactory flow of secretion has been established, it will be maintained at a constant level of volume, free and total acidity for periods as long as 10 hours.

Discussion.

A definite relation between the CO_2 content of the plasma and gastric secretion is seen in the group of nerve stimulation experiments. Hyperventilation causes a cessation of gastric secretion. That this cessation is related in some way to the coincident lowering of the CO_2 content of the plasma is indicated by the restoration of the flow of gastric juice by the increase of the CO_2 content of the inspired air. The mechanism of the above effects is dependent upon numerous factors. We do not feel that a complete explanation is possible at the present time. We believe, however, that there are certain of these factors which can be excluded. Hyperventilation has been found by previous authors to have a variety of effects upon the vaso-motor system and upon the chemical composition of the blood. Dale and Evans [1922] found a marked lowering of blood-pressure and considerable shock in cats under ether anæsthesia. McDowall [1930] showed that this fall in blood-pressure did not always occur under chloralose. Lowering of blood-pressure if due to splanchnic vaso-dilatation should favour secretion. The blood-pressure effect was not marked in our experiments, being at most 30–40 mm. of mercury. The initial pressure was high owing to the proportions of the chloralose-urethane mixture used. Also, in several experiments nerve stimulation has maintained satisfactory secretion with blood-pressures lower than those produced by hyperventilation. The changes in systemic blood-pressure do not, therefore, appear to be the causative factor in the gastric effect. This does not, however, exclude the effect of variations in blood flow through the gastric mucosa. Vasoconstriction of blood vessels might be expected to cause a diminution in secretion. It has been indicated by Lim and Necheles [1927] that within limits the rate of gastric secretion is independent of the blood flow through the stomach. Furthermore, hyperventilation has been shown to dilate the splanchnic vessels [Dale and Evans, 1922], and this occurs even under chloralose [McDowall, 1930]. We have not studied blood flow in our preparations but feel that its influence may be disregarded in view of the findings quoted above. Mechanical effects of hyperventilation are excluded by the maintenance of a constant rate of ventilation throughout the whole experiment, both during cessation of secretion and during its restoration by CO_2.

The changes in blood electrolytes occurring under hyperventilation have been extensively studied [Henderson and Haggard, 1918; Collip and Backus, 1920; Grant and Goldman, 1920; Davies and

others, 1920; Anrep and Cannan, 1923; Haldane and others, 1924]. The experiments have been made almost wholly upon human subjects. The main changes which take place are a lowering in the CO_2 content of the blood, a rise in pH and in lactic acid content, a shift of water and chloride from corpuscles to plasma. Dependent upon the change in pH there is an alteration in the dissociation curve of hæmoglobin. Other electrolytes such as phosphates and sulphates also participate in the shift from corpuscles to plasma. The blood picture in our experiments conforms to these findings (Table II). The increased loss of CO_2 through

TABLE II. Changes in blood constituents under hyperventilation of a 5 p.c. CO_2 oxygen mixture.

	CO_2 plasma m.Eq./l.	pH	Lactic acid blood m.Eq./l.	Cl plasma m.Eq./l.	Cl blood m.Eq./l.
Before hyperventilation	18 3	7·24	3·67	106·4	86·4
After 50 min. Hyperventilation 76 per min.	9·5	7·60	8·22	105·1	84·2
40 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	—	—	—	—	—
After 30 min. Hyperventilation, air only	9·3	7·57	10·1	105·0	81·7
After 30 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	18·9	7·10	3·81	104·9	85·3
After 30 min. natural respiration, air only	15·5	7·34	11·9	103·6	83·1

	Cl corpuscles m.Eq./l.	Plasma proteins g. p.c.	Inorganic phosphorus mg./100 c.c.	Total base serum m.Eq./l.	Cell volume
Before hyperventilation	56·3	8·05	6·66	126·2	40
After 50 min. Hyperventilation 76 per min.	50·7	8·65	3·46	131·4	39
40 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	—	—	—	—	—
After 30 min. Hyperventilation, air only	44·2	9·04	4·70	132·2	39
After 30 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	58·3	8·28	7·20	138·6	42
After 30 min. natural respiration, air only	54·1	—	8·33	159·6	41

Dog 13. Wt. 14 kg. Histamine (4 mg.) injected hourly. Secretion of gastric juice maintained throughout the experiment. Inorganic phosphates determined by the method of Fiske and Subbarow [1925] and total base by that of Stadie and Ross [1925]. Corpuscle chlorides were obtained by difference. m.Eq./l. = milli-equivalents per litre.

the lungs with the consequent fall in the blood CO_2 content is responsible for the other changes in blood electrolytes which are of a compensatory nature.

That the lowered CO_2 content rather than the raised pH is the factor involved in the gastric effect is indicated by the experiments in which initial acidosis with a lowered CO_2 and a lowered pH prevented the production of secretion. The subsequent injection of sodium bicarbonate raised the CO_2 content of the plasma, raised the pH and caused secretion to commence. The above-mentioned experiments of Szilard [1930] and others also indicate that a high pH produced by administration of sodium bicarbonate does not inhibit but rather favours the secretion of acid gastric juice. In the case of hyperventilation, no secretion was obtained when there was a low CO_2 content and a high pH, whereas in the case of initial acidosis no secretion was obtained when there was a low CO_2 and a low pH. Raising the CO_2 content and lowering the pH induced secretion in the former instance, and raising the CO_2 and raising the pH induced it in the latter. Hence, one may conclude that the observed changes in secretion are not directly due to the changes in plasma pH nor to concomitant alterations in the oxygen dissociation curve of hæmoglobin. The experiments with cyanide also indicate that the oxygen tension in the secreting cells may be varied to some degree at least without effect on secretion.

Apperly and Crabtree [1931] have attempted to separate the effects of H_2CO_3 and of "HCO_3." The former they regard as controlling the amount of gastric acidity and the latter its concentration. We feel that neither their results nor ours justify a definite decision on the relative importance of CO_2 present as H_2CO_3 and as "HCO_3." In this connection we may cite the experiment described above where the secretion obtained by vagal stimulation was temporarily increased in free HCl concentration by the injection of hydrochloric acid, while H_2CO_3 concentration increased at the expense of "HCO_3." In the experiments with hyperventilation, the changes in H_2CO_3 are naturally greater than the changes in "HCO_3"; on the other hand, the induction of secretion and a rapid rise in the concentration and amount of free HCl by injecting sodium bicarbonate into dogs in which spontaneous acidosis had prevented the response to vagal stimulation or to histamine, are accompanied by great increases in "HCO_3" with only slight changes in H_2CO_3 concentration (calculated from the Henderson-Hasselbalch equation).

The lowered plasma CO_2 content may conceivably act either directly upon the nerve or its terminations or alternatively upon the chemical mechanism necessary for the formation of gastric secretion.

Just how changes in plasma CO_2 content affect nerve conduction

and nerve endings we are unable to state. In our experiments when secretion had diminished under hyperventilation, there was no change in the cardio-inhibitory action of the vagus. It is, however, possible that the nerve endings in the stomach might be affected. The possible effect of lowered CO_2 content on the liberation and destruction of a "vagus substance" should also be considered.

On the view that the action of CO_2 is a chemical one rather than an action on nerve sensitivity, its effect may be a direct one in the reaction for the formation of hydrochloric acid in the parietal cells or an effect upon the ionic equilibria between blood plasma and tissue fluids or between tissue fluids and parietal cells. The lowered CO_2 content may prevent transfer of chloride to the mucosa or parietal cells. This retention of chloride tends to take place to balance the fixed base liberated when the bicarbonate ion content of the blood or tissue fluid is lowered. In connection with this it may be noted that recently Babkin [1932] and Webster obtained a gastric secretion introducing CO_2 gas into the stomach of dogs with different permanent gastric fistulæ.

In the group of histamine experiments it will be observed that the results differ decidedly from those of the nerve stimulation experiments. When histamine secretion was established hyperventilation had little inhibitory effect, even though blood conditions were similar to those of the nerve experiments. The inhalation of CO_2 is seen to tend to inhibit histamine secretion. While this cannot be explained, we feel that it does not invalidate the main argument. In several cases of initial acidosis with low CO_2 and low *p*H, no secretion was obtained with histamine stimulation and as in the nerve experiments, it was activated when the CO_2 and *p*H were raised following the injection of sodium bicarbonate. On the other hand, when acidosis is produced by the administration of acid during histamine secretion, no inhibitory effect is observed. The absence of effect after histamine secretion has been established does not invalidate a chemical interpretation of the essential part played by CO_2 in the formation of HCl. It is possible that the level of CO_2 required for secretion may be related to the strength of the stimulus producing that secretion. It is impossible to lower the CO_2 content of the blood and of the tissues beyond a certain value. If the stimulus is an extremely powerful one (such as histamine) the parietal cells may be capable of utilizing the remaining CO_2 which they are incapable of doing under a weaker and more physiological one (such as vagal stimulation). The absence of secretion under conditions of acidosis existing prior to the injection of histamine indicates that a lowered

CO_2 content is capable of inhibiting the action even of this powerful stimulant.

The clinical application of these results appears to us to be of some importance. Apperly and Crabtree [1931] have indicated some conditions in which the lowering of gastric secretion may be correlated with lowered sodium bicarbonate in the blood. We would draw attention to some considerations which may be of significance in any clinical application. We have found that the level of arterial CO_2 content at which inhibition of secretion takes place is about 30 vol. p.c. This is true whether the lowering of the CO_2 is due to hyperventilation or to acidosis. This corresponds to a venous CO_2 content of about 36 vol. p.c. In one experiment where both CO_2 content and the ordinary CO_2 combining power on separated plasma were done, the initial values were: CO_2 content, 34·8 vol. p.c., and the capacity or combining power, 39·5 vol. p.c.; after secretion was well established the values were: content 43·3 and capacity 45·1. Under hyperventilation when secretion had ceased and the CO_2 content had been lowered to 25·4, the capacity was only lowered to 35·8. After the administration of CO_2 the content was 41·4 and the capacity 40·5. These figures are presented to indicate that the CO_2 combining power of the separated plasma is not a suitable index in the case of hyperventilation of the relations between CO_2 and gastric secretion. Complete inhibition of secretion in this experiment took place when the arterial CO_2 combining power was 36 vol. p.c. This corresponds to a venous CO_2 combining power of about 42. A definite decrease in secretion might, therefore, be expected even if the CO_2 combining power were only slightly lowered. Such a slight lowering is not uncommon with mild acidosis and with hyperventilation and might easily influence the character of gastric secretion. It would be difficult to detect these changes in the case of hyperventilation by the use of the ordinary CO_2 combining power determination in venous blood. Changes in CO_2 alveolar air tension and hence of CO_2 content of the blood during sleep and on waking [Leathes, 1919; Endres, 1922] and during digestion [Porges, Leimdorfer and Markovici, 1911; Dodds and Bennett, 1921] do take place under physiological conditions.

It is possible on the other hand that a raised CO_2 content sensitizes the parietal cells so that they respond more readily to a weak stimulus. This may be of importance in cases of hypersecretion where apparently a stimulus which in normal individuals would initiate a secretion of average acidity produces an excessive secretion with abnormally high acid content. For some years there has been a controversy as to the

usefulness of sodium bicarbonate therapy in the treatment of gastric ulcer, with hyperacidity. Our results would tend to support those who hold that its use though producing a temporary neutralization is followed by a secretion of greatly augmented acidity.

Summary.

1. Gastric secretion produced by vagal stimulation in dogs under chloralose-urethane anæsthesia is inhibited by hyperventilation. It is restored by raising the CO_2 content of the inspired air, though the hyperventilation is maintained at the same rate as before.

2. Secretion is also inhibited by the injection of acid intravenously and by the occurrence of acidosis prior to the stimulation of the nerves.

3. The factor involved in the effect of hyperventilation and acidosis on gastric secretion is the lowering of the CO_2 content of the plasma rather than the accompanying changes in plasma *p*H.

4. Gastric secretion in response to vagal stimulation is inhibited when the CO_2 content of the arterial plasma falls below 30 vol. p.c.

5. Secretion in response to injections of histamine is also inhibited *by the occurrence of acidosis prior to the commencement of secretion but not by acidosis or hyperventilation produced after secretion is* established.

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THE INFLUENCE OF THE PARATHYROID ON THE METABOLISM OF CREATINE AND PHOSPHORIC ACID.

Part I. Phosphate excretion after injections of creatine and parathormone.

By MARION BROWN AND C. G. IMRIE.

Part II. Regulation of creatine phosphate after thyroparathyroidectomy, etc.

By C. G. IMRIE AND CONSTANCE N. JENKINSON.

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PART I.

THERE is evidence that the administration of creatine to cats is followed by a temporary reduction in the output of phosphates by the kidney. The effect appears to be determined not so much by the amount of creatine given as by the quantity retained in the body, since the retention of creatine in amounts equivalent to 80 mg. or more per kg. of body weight is associated with a greater decrease in the excretion of phosphate than occurs when the amount retained is less [Brown and Imrie, 1931 *a*]. There is also evidence that the concentration of total acid-soluble phosphorus in the muscles tends to be high when the content of creatine is high normally or has been raised experimentally [Brown and Imrie, 1931 *b*]. It is conceivable therefore that creatine, so administered, combines with phosphoric acid and is taken up by the muscles possibly as creatine phosphate.

It is well known that the parathyroid gland influences the metabolism of phosphorus and that creatinuria occurs in animals deprived of their parathyroids. In view of this, some experiments have been carried out to test the effect of parathyroid extract on the reaction just referred to between creatine and phosphoric acid.

Experimental procedure.

Decerebrate cats were used. Creatine in amounts corresponding to 200 mg. per kg. of body weight, dissolved in normal saline 85 c.c./kg., was injected intravenously at a uniform rate. The injection time was 3 hours. Parathormone (Collip) was given in doses of 20 units as indicated in the charts. The urine was collected each half hour. The phosphates were determined by Brigg's method, the creatine by the method of Folin. In all experiments an hour elapsed after decerebration before the injections were begun.

Fig. 1 comprises the results of four experiments where creatine was injected; in three of these parathormone had been given and in one it had not. The difference is striking. With creatine alone the output of phosphates is diminished for a brief period only; with parathormone it is in total abeyance for $4\frac{1}{2}$–6 hours, *i.e.* to the end of the period of observation. This effect is manifest in $1\frac{1}{2}$, 2 and 5 hours respectively in the three experiments. Only one experiment in which creatine alone was given is included, as details of similar experiments are to be found in a former paper [Brown and Imrie, 1931 *a*].

In Fig. 2 are shown the results with single doses of parathormone (20 units), on one occasion administered 18 hours before the injection of creatine was begun, on the other immediately before. In the former instance (Exp. 11) the effects resemble those with two doses, the phosphates are practically gone from the urine in $1\frac{1}{2}$ hours and have not returned 11 hours later; in the latter (Exp. 7) the phosphates disappear for 3 hours and then gradually return. Two control experiments with parathormone and saline are included.

In one of these (Exp. 4) where no diuresis occurs the output of phosphates is maintained, and in the other (Exp. 5) with a moderate diuresis it rises with the onset of the diuresis and then falls somewhat.

Certain points arise from these experiments upon which comment may be made. The reduction in the output of the phosphates by the kidney associated with the administration of creatine is more pronounced when parathyroid extract has been given some hours before the creatine. In one experiment where it was given immediately before the creatine it did not appear to exert any influence (Exp. 7). The doses of parathormone given were large, perhaps larger than necessary, but they do not appear to affect the power of the kidney to excrete water, phosphate or creatine. Collip, Clark and Scott [1925] observed that massive

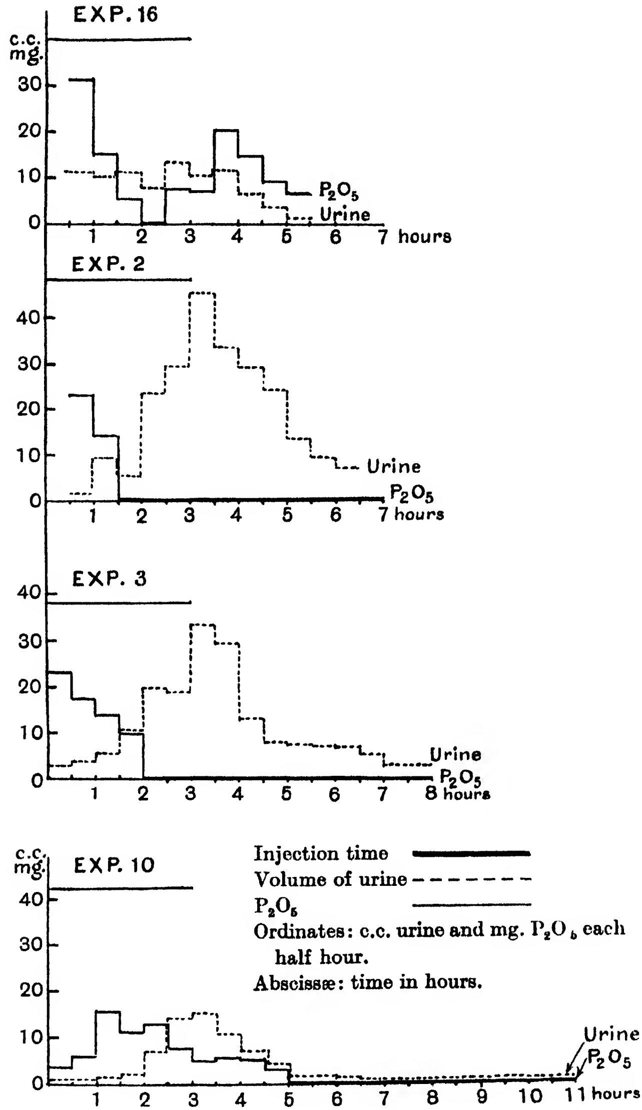

Fig. 1.

Exp.	16	2	3	10
Creatine injected mg./kg.	200	200	200	200
Creatine retained mg./kg.	71	96	95	91
Volume of saline injected, 85 c.c./kg.	252	204	162	213
Total volume of urine, c.c.	85	230	173	66
Parathormone, units injected:				
18 hours before exp.	—	20	20	20
Immediately before exp.	—	20	20	20

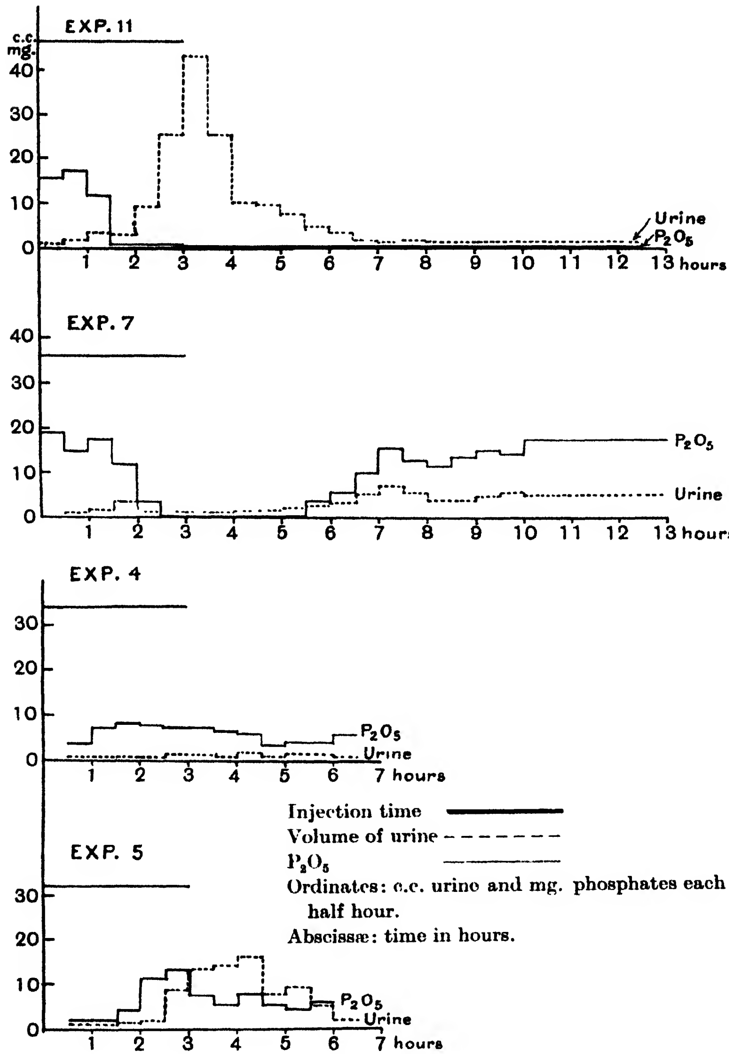

Fig. 2.

Exp.	11	7	4	5
Creatine injected mg./kg.	200	200	—	—
Creatine retained mg./kg.	87	50	—	—
Volume of saline injected, 85 c.c./kg.	183	227	174	174
Total volume of urine, c.c.	153	95	6	75
Parathormone, units injected:				
18 hours before exp.	20	—	20	20
Immediately before exp.	—	20	20	20

single doses of the extract may be given to dogs without injurious effect, and that cats are more resistant to parathormone than dogs. It is unlikely therefore that the results are due to a toxic action of the parathormone on the kidney.

The striking diuresis which occurred in three of the experiments with parathormone and creatine is of interest. During the course of two of these the volume of the urine was greater than that of the fluid injected, and in a third it was only a little less. The disappearance of the phosphates from the urine was more abrupt in these instances. In Exp. 7, however, where it is reasonable to suppose a creatine effect alone was obtained as there was not sufficient time for the parathormone to act, no diuresis occurred within the first 6 hours, but the phosphates disappeared from the urine and reappeared 3½ hours later. This subject has not been pursued further.

Since the results suggest that the parathyroid gland is concerned with the metabolism of creatine and phosphorus in the body, it was thought desirable to seek more direct evidence of such a relationship by a study of the creatine phosphate in the muscles under similar experimental conditions. Accordingly, a short series of experiments were carried out in which determinations were made of the creatine phosphate in the muscles of cats before and after the injection of creatine, with and without parathormone.

Decerebrate cats were used. After decerebration the nerves to the hindlimbs were cut to eliminate the influence of such factors as decerebrate rigidity and muscular movements. The preparations were left an hour to recover from the effects of the anæsthetics and the muscular movements associated with the induction of anæsthesia. Portions of muscles were removed for examination before the injections were begun, immediately after they were completed and 1 hour later. Creatine phosphate was determined by the Eggletons' method [P. and E. G. Eggleton, 1929]. Creatine, 200 mg./kg. dissolved in 85 c.c./kg. of normal saline, when given was injected during 3 hours. In two experiments saline only was administered, in two creatine alone, in one saline after parathormone, and in four others creatine after parathormone. One dose of the extract, 20 units, was given on these occasions. The figures for these experiments are set out in Table I.

There is a tendency for the concentration of the creatine phosphate to rise to a higher level in those instances where creatine and parathormone were both given than in the others where each was given separately or saline without either of them. The difference is not very

TABLE I. Creatine phosphate in the muscles of cats.

	I	II	III	IV
Saline	71·3	75·6	66·6	70·9
,,	68·5	66·8	73·6	69·3
Creatine	60·6	62·4	60·5	74·5
,,	63·6	63·0	68·4	71·0
Parathormone and saline	58·1	55·9	65·7	58·9
Creatine and parathormone	61·6	63·2	71·0	81·2
,,	57·8	67·4	69·6	81·7
,,	63·7	68·4	78·8	72·7
,,	69·7	70·2	83·9	72·4

Figures express mg. of phosphorus as creatine phosphate per 100 g. of muscle.
I and II, muscles removed before injections, III at the end of the injection and IV 1 hour later.
Saline 85 c.c./kg., creatine 200 mg./kg. dissolved in 85 c.c./kg., given in 3 hours.
Parathormone 20 units, 18 hours before the experiments.

great, but it is quite possible that the high values represent the maximal capacity of the muscles for creatine phosphate.

When parathormone is administered alone without the subsequent injection of any fluid, it does not appear to influence the concentration of creatine phosphate or the other forms of phosphorus in the muscles. This is shown in the figures of Table II. They are the values obtained from the muscles examined in the experiments just described before the injections of creatine or saline were begun.

TABLE II.

	Phosphorus per 100 g. of muscle as						
	Creatine phosphate	Orthophosphate	Soluble ester	Pyrophosphate	Insoluble ester	Total phosphate	Water p.c.
Control	71·3	24·4	20·2	35·9	30·3	182	—
,,	68·5	25·1	4·5	33·5	35·8	167	74·7
,,	63·6	27·4	10·3	39·1	28·4	169	74·6
,,	62·8	28·3	14·2	40·0	44·0	190	74·1
,,	60·6	34·0	5·2	37·5	37·3	175	76·7
Averages	65·4	27·8	10·9	37·2	35·2	177	75·0
Parathormone	68·4	25·5	11·3	30·0	32·5	168	73·4
,,	67·6	33·1	13·8	38·9	32·6	186	72·5
,,	64·8	33·2	21·2	29·5	50·6	199	74·8
,,	61·6	32·6	17·6	36·4	45·7	194	74·0
,,	58·1	28·6	23·0	34·2	40·0	184	74·3
,,	57·8	30·0	18·8	—	—	168	73·5
Averages	63·1	30·5	17·6	33·8	40·3	183	73·7

Parathormone, 20 units administered 18 hours before.

As seen in the table the average figures for the creatine phosphate in the two groups, the one where parathormone had been given, the other where it had not, are practically the same. Similar comment may

be made regarding the other forms of phosphorus except the soluble esters. In two of the controls very low values for this form of phosphorus were found.

Table III. Thyroparathyroidectomized cats.

	Milligrams of phosphorus per 100 g. of muscle as						
	Creatine phosphate	Orthophosphate	Soluble ester	Pyrophosphate	Insoluble ester	Total phosphate	Water p.c.
I. *Decerebrated at* 10.30 *a.m.*:							
Muscles removed at							
11 a.m.	30·5	73·4	4·5	40·8	18·2	167	74·3
12 noon	25·7	82·9	5·8	38·1	19·4	172	73·2
II. *Decerebrated at* 10.30 *a.m.*:							
Muscles removed at							
11.10 a.m.	34·1	73·7	3·9	19·6	13·9	145	75·7
12 noon	27·2	78·3	9·7	31·9	12·8	160	75·4
III. *Chloralose at* 10.45 *a.m.*:							
Muscles removed at							
11.15 a.m.	35·7	61·4	16·8	41·3	30·5	186	—
12.30 p.m.	32·9	63·1	17·8	41·5	28·4	184	—
2.30 p.m.	28·1	76·6	12·8	52·4	32·1	202	—
4.30 p.m.	34·4	70·0	14·9	51·2	31·0	201	—

In Table III are shown figures which were obtained from the muscles of three cats which had developed tetany following thyroparathyroidectomy. The nerves to the hindlimbs were cut after decerebration or the administration of ether for the injection of chloralose, and muscles were removed for examination at the times indicated in the table. The concentration of creatine phosphate is low, that of the orthophosphates high, as compared with normal values, and in Exp. 3 the creatine phosphate has not increased during the period of observation. The orthophosphates have remained much higher than normal. The influence of the parathyroid on the resynthesis of creatine phosphate will be considered in the next paper.

Conclusions.

1. The temporary reduction in the output of phosphates by the kidney, which follows the administration of creatine, is rendered more pronounced by parathyroid extract (Collip's parathormone).

2. Following the injection of creatine, somewhat higher values for the creatine phosphate in the muscles are found in cats which have received parathyroid extract previously than in those which have not.

3. Parathyroid extract as administered in the experiments described does not appear to influence the concentration of creatine phosphate or the other forms of phosphorus in the muscles of normal cats.

4. In three thyroparathyroidectomized cats which had developed tetany the concentration of creatine phosphate was low, that of the orthophosphates high, as compared with normal muscles, and remained so throughout the period of observation.

PART II.

The results set out in Part I suggest that the parathyroid gland may be concerned with the metabolism of creatine phosphate in the muscles. It is conceivable that the resynthesis of creatine phosphate is under hormone control and that the parathyroid glands provide the hormone. In order to test this hypothesis some observations have been made on the rate of resynthesis of creatine phosphate following stimulation of the muscles in normal and thyroparathyroidectomized cats.

Experimental procedure.

Cats, decerebrated or anæsthetized with chloralose were used. Immediately after decerebration or the induction of ether anæsthesia for the chloralose injections, the crural and sciatic nerves in both hindlimbs were cut. The animals were left an hour in order that they should recover from the effects of the ether and from the changes in the creatine phosphate arising from the muscular movements associated with the induction of anæsthesia. A portion of muscle was then removed for examination and the peripheral ends of the divided nerves were faradized for 20 min. The two electrodes employed were attached to the same induction coil. Determinations were made on muscles removed immediately after stimulation, at 15 min. intervals for the first hour and at the end of the second and third hours. The Eggletons' method [1929] was followed throughout except that the muscles were killed in liquid air. It was found that the muscles were pulverized more readily when this procedure was adopted. The concentrations of the different forms of phosphorus were determined, but in this paper only the creatine phosphate and the orthophosphate will be considered.

Results.

In Table I are set out figures for the creatine phosphate before and after stimulation, obtained from the muscles of seven normal cats, two decerebrated and five under chloralose. The results show quite clearly

Table I. Creatine phosphate in the muscles of normal cats before and after stimulation. Figures express creatine phosphate as mg. phosphorus per 100 g. muscle.

No.	Wt. in kg.	D or C	Before stimulation	Zero	15 min.	30 min.	45 min.	60 min.	2 hr.	3 hr.
4	3	D	75·3	34·0	48·5	52·7	66·0	71·7	72·2	—
3	3	D	69·5	32·3	39·2	52·7	64·3	68·9	68·7	—
9	1·8	C	64·1	30·5	46	52·2	56·3	53	61·4	63·3
10	1·8	C	62·8	30·5	39	44·4	51·9	56·6	59	—
6	1·9	C	60·5	31·8	44·8	41·6	44·6	54·5	60·2	—
8	1·9	C	54	27·8	37·2	45	42·7	41·5	41	49
7	1·5	C	44·2	27·6	35·2	34·4	44	47·5	50·6	52
Averages			61·5	30·7	39	44·5	51·9	56·6	62·1	—

D, decerebrate; C, chloralose.

that the concentration of creatine phosphate is reduced by stimulation, its resynthesis is gradual and that from 45 to 60 min. elapse before the original value is restored.

In Table II, where figures for the orthophosphates in these experiments are recorded, changes of an opposite character appear. The concentration of the orthophosphates increases as a result of stimulation

Table II. Orthophosphates in the muscles of normal cats before and after stimulation. Figures express orthophosphates as mg. phosphorus per 100 g. muscle.

No.	Wt. in kg.	D or C	Before stimulation	Zero	15 min.	30 min.	45 min.	60 min.	2 hr.	3 hr.
4	3	D	44·9	74·2	62·9	55·6	46	40·7	53·9	—
3	3	D	45·3	75·4	74·8	60·5	48	42·5	39	—
9	1·8	C	34·4	73·1	46·3	42·9	36·3	46·3	34·2	39
10	1·8	C	29·8	55·5	51·9	42·9	43·3	35	32·5	34·1
6	1·9	C	31·8	58	40·7	49·7	48·2	40·5	37·3	—
8	1·9	C	41	82·2	74·1	59·5	61·2	45·1	50·5	45·1
7	1·5	C	31·2	62·6	63·5	49·5	46·2	42·8	37·3	31·2
Averages			36·9	68·7	57·7	51·5	47	41·8	40·7	37·3

D, decerebrate; C, chloralose.

and then decreases at a rate corresponding to that at which the creatine phosphate is resynthesized. In Chart 1 the average figures for the creatine phosphate and the orthophosphates in this group of experiments are plotted in the form of a graph. The reciprocal nature of the changes and the degree to which they correspond are clearly shown.

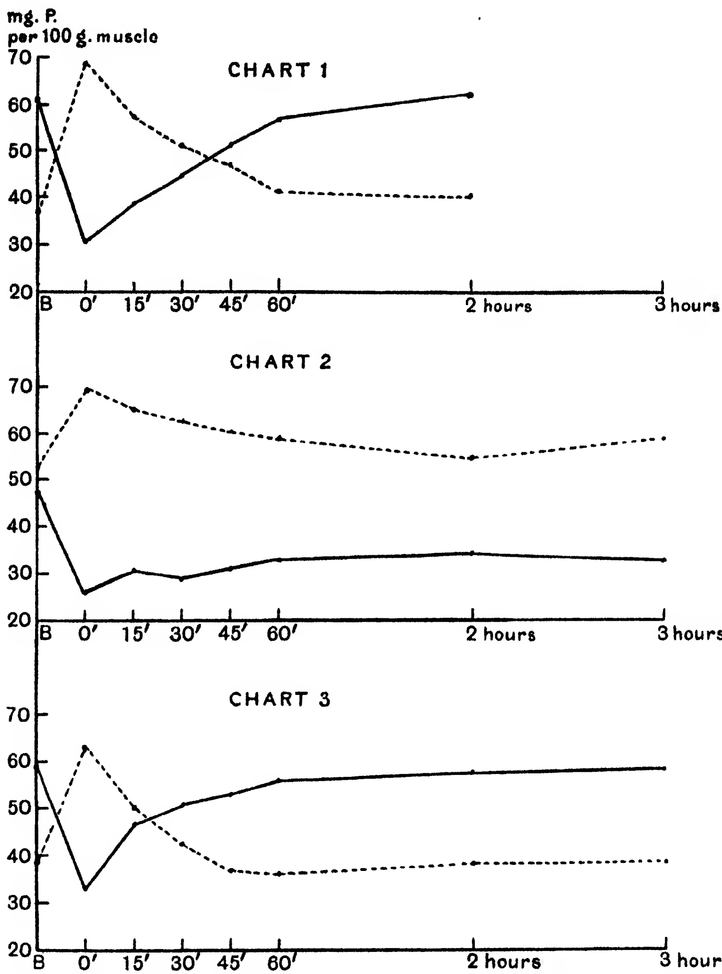

Chart 1. Creatine phosphate and orthophosphates before and after stimulation in the muscles of normal cats.

Chart 2. Creatine phosphate and orthophosphates before and after stimulation in the muscles of thyroparathyroidectomized cats.

Chart 3. Creatine phosphate and orthophosphates before and after stimulation in the muscles of thyroparathyroidectomized cats treated with parathormone.

Ordinates: mg. phosphorus per 100 g. muscle. Abscissæ: time, B, before stimulation; O, immediately after stimulation. Continuous line, creatine phosphate. Interrupted line, orthophosphates.

But in animals which have developed tetany following thyroparathyroidectomy quite a different result is obtained. Values for the creatine phosphate and the orthophosphates in the muscles of twelve such cats are set out in Tables III and IV respectively, and graphs of the averages

TABLE III. Creatine phosphate in the muscles of thyroparathyroidectomized cats before and after stimulation. Figures express creatine phosphate as mg. phosphorus per 100 g. muscle.

No.	Wt. in kg.	D or C	Days after operation	Before stimulation	Zero	15 min.	30 min.	45 min.	60 min.	2 hr.	3 hr.
1	2	D	3	65·4	28·1	28·4	31·7	36·2	34·6	33·1	—
14	1·6	C	2	58·5	34·8	36·2	37·2	32·8	32·4	42·0	—
13	0·9	C	1	54·5	36·6	42·3	41·0	41·7	39·0	39·2	—
4	2·4	D	12	54·1	25·8	32·2	28·2	30·5	38·2	37·0	—
9	1·6	C	2	52·6	25·4	27·8	28·0	28·5	32·8	35·4	—
2	2·2	D	6	49·5	23·1	38·0	32·7	41·6	46·8	42·4	—
11	1·5	C	2	48·6	23·5	28·0	31·5	38·0	35·1	28·3	30·8
8	2	C	2	44·9	24·6	38·0	32·7	41·6	46·8	42·4	—
26	1·2	C	1·5	44·4	27·3	33·2	29·3	31·5	33·0	30·6	32·4
25	1	C	6	40·8	30·5	32·2	28·5	27·8	26·8	35·2	34·8
5	2·4	D	5	37·8	20·0	22·6	17·9	16·0	17·4	13·1	—
6	1·5	D	1	23·2	12·2	12·7	11·6	11·3	12·0	—	—
	Averages			47·8	26·0	31·0	29·2	31·5	32·9	34·4	—

D, decerebrate; C, chloralose.

Cat No. 4 developed tetany on the fifth day, was then given parathormone for 2 days and then left until tetany was again developed on the twelfth day.

Cat No. 25 developed tetany on the second day, was then given parathormone for 2 days and then left until tetany was again developed on the sixth day.

Zero, immediately after stimulation.

TABLE IV. Orthophosphates in the muscles of thyroparathyroidectomized cats before and after stimulation. Figures express orthophosphates as mg. phosphorus per 100 g. muscle.

No.	Wt. in kg.	D or C	Before stimulation	Zero	15 min.	30 min.	45 min.	60 min.	2 hr.	3 hr.
1	2	D	74·2	89·9	81·5	65·3	64·9	63·2	54	84·6
14	1·6	C	46·2	65·8	64·4	58	56·2	57·6	54·4	58·6
13	0·9	C	53	62·4	56·2	55	47·4	49·5	46·7	50·1
4	2·4	D	54·3	76·4	63·3	58·8	57·4	51·7	51·9	56·4
9	1·6	C	54·2	54·8	48	51·2	53·6	50·8	47	40
2	2·2	D	35·7	61·2	53·7	63	52·4	49·2	60·5	—
11	1·5	C	44·8	52·4	53	46	55	52·7	52·5	55·6
8	2	C	49·5	55·8	58·8	58·4	53·8	50·2	56·7	48·2
26	1·2	C	33·7	55·6	50	54	51·2	49·5	49·9	59·4
25	1	C	53·2	60	59·4	58·8	53·5	54·5	51·3	55·8
5	2·4	D	48·8	74·3	82·9	74·8	65·7	75·2	87·5	87·5
6	1·5	D	89·2	127	114·5	112·5	109·8	110·9	—	—
	Averages		53	69·6	65·5	63	60·1	59·6	55·7	59·6

D, decerebrate; C, chloralose.

Cats Nos. 4 and 25, see footnote Table III.

Zero, immediately after stimulation.

are plotted in Chart 2. The average initial value of the creatine phosphate tends to be lower, that of the orthophosphates higher than in normal animals. The concentration of the creatine phosphate is reduced to a slightly lower level by stimulation, and its resynthesis takes place at a very much slower rate than in the normal cats. The changes in the orthophosphates are reciprocal in nature and correspond in degree to those observed in the creatine phosphate.

In a third group of five cats thyroparathyroidectomy was performed and after tetany had developed parathormone was given and the animals allowed to recover. Similar observations were then carried out. Figures for this group are shown in Tables V and VI and the graph in Chart 3.

TABLE V. Creatine phosphate in the muscles of thyroparathyroidectomized cats treated with parathormone. Figures express creatine phosphate as mg. phosphorus per 100 g. muscle.

No.	Wt. in kg.	Days after operation *	Days after operation †	Before stimulation	Zero	15 min.	30 min.	45 min.	60 min.	2 hr.	3 hr.
20	2	2	3	60·8	33·7	44·4	46·9	56·6	55·6	57·6	60·7
17	1·1	5	2	60·3	27·2	43·9	52·8	60·0	58·7	59·0	58·1
19	1·1	1	2	59·5	34·4	47·6	52·7	49·0	55·5	60·5	60·3
23	1	4	4	57·9	36·6	48·6	51·1	49·6	53·7	58·5	58·6
21	1·2	1	2	56·8	37·1	47·2	49·1	49·4	56·7	54·5	54·4
Averages				59·1	33·8	46·3	50·5	52·9	56·0	58·0	58·4

* Days after operation before tetany appeared.
† Number of days of treatment, parathormone 10 units twice daily.
Zero, immediately after stimulation.
Chloralose anæsthesia.

TABLE VI. Orthophosphates in the muscles of thyroparathyroidectomized cats treated with parathormone. Figures express orthophosphates as mg. phosphorus per 100 g. muscle.

No.	Wt. in kg.	Days after operation *	Days after operation †	Before stimulation	Zero	15 min.	30 min.	45 min.	60 min.	2 hr.	3 hr.
20	2	2	3	38·4	57	50·7	39·8	38·4	40·8	39·9	35·1
17	1·1	5	2	42·4	93	62·6	45·6	33·4	37·1	40·7	40·2
19	1·1	1	2	42·3	61·9	49·5	46·7	41·5	38·9	39·8	42·6
23	1	4	4	34	47·7	40·8	40·5	34·8	31·5	35·2	35·1
21	1·2	1	2	34·3	58·4	46·4	39·7	37·7	37·8	39·0	39·6
Averages				38·3	63·6	50·0	42·4	37·1	36·2	38·9	38·5

* Days after operation before tetany appeared.
† Number of days of treatment, parathormone 10 units twice daily.
Zero, immediately after stimulation.
Chloralose anæsthesia.

It is apparent that the initial values for the creatine phosphate and the orthophosphates agree with the average figures for normal cats and that the rate of resynthesis of the creatine phosphate and the decrease of the orthophosphates after stimulation is more rapid in the early stages than in normal cats.

Discussion.

The average results of groups, rather than of individual members of a group, have been considered in this paper. In a work of this nature, where so many unknown factors exist, the results of groups are less likely to mislead. All the cats which have been examined in each group are included in the tables; there has been no selection of results.

In the group of normal cats, the initial values for the creatine phosphate show a certain range of variation, 75·3–44·2 mg. The latter figure is unusually low. In a series of more than 40 cats, in which the nerves to the limbs were not cut and where determinations of the creatine phosphate were made under somewhat different conditions but of such a nature as would tend to decrease the creatine phosphate, on one occasion only was a value below 50 mg. observed. More uniformity appears in the figures immediately after stimulation, and according to the average values the concentration of the creatine phosphate has been reduced approximately 50 p.c. by this procedure. The muscles were removed as rapidly as possible after the stimulation, but one or two minutes were required to do this, and as resynthesis of the creatine phosphate was no doubt taking place during this time, the actual concentration of this form of phosphorus at the end of the stimulation was somewhat lower than our figures indicate. On the other hand mechanical stimulation of the muscle during its removal would diminish the creatine phosphate.

In the twelve thyroparathyroidectomized cats the initial values for the creatine phosphate show a wider range of variation and the average value is lower than in the normal. In two instances, Exps. 2 and 8, resynthesis of the creatine phosphate to the original level has taken place. It is not surprising that this should happen in a series of twelve cats, as cats frequently possess accessory parathyroid glands which are not removed when the thyroid and parathyroids in the neck are taken out. In the later stages of this work cats were rejected which did not show more definite signs of tetany than were observed in these two animals.

Conclusions.

1. The creatine phosphate in the muscles of normal cats is reduced by stimulation to approximately 50 p.c. of the original concentration. Resynthesis appears to be gradual and requires from 45 to 60 min. for its completion. The orthophosphates show changes of a reciprocal nature.

2. The creatine phosphate in the muscles of thyroparathyroidectomized cats tends to be lower than in normal ones. It is reduced by stimulation to a slightly lower level and the rate of resynthesis is very much slower than that observed in the normal cats, in some instances no resynthesis has taken place in 2–3 hours. The orthophosphates show reciprocal changes.

3. The creatine phosphate and the orthophosphates in the muscles of thyroparathyroidectomized cats treated with parathormone resemble the normal. After stimulation, however, the resynthesis of the creatine phosphate and the decrease of the orthophosphates are more rapid in the early stages than in the normal cats.

4. These results support the original hypothesis that the parathyroid glands are concerned with the metabolism of creatine phosphate in the muscles and that the active principle is contained in parathormone.

It is a pleasure to acknowledge our thanks to Prof. Leathes for his interest and advice.

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THE INFLUENCE OF TOXÆMIA ON CARBOHYDRATE METABOLISM.

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CLAUDE BERNARD was probably the first to realize the harmful effect of infection on a diabetic patient's carbohydrate metabolism, and of recent years this phenomenon has become widely recognized by the clinician. The tendency of sepsis or febrile infections to cause further impairment of glucose tolerance usually necessitates an increment in the insulin dosage, and occasionally results in the production of a so-called "insulin resistant" state, in which relatively large doses of insulin fail to control the hyperglycæmia. The seriousness of such disturbances in diabetic individuals can be appreciated when it is realized that infection, even in non-diabetic individuals, may provoke a state of diminished glucose tolerance.

Experimentally, it is possible to produce a somewhat analogous condition in laboratory animals, and various explanations have been based on such experiments to explain the condition found in man.

Sweeney [1928] assumed that infection caused a diminished output of endogenous insulin, but most observers do not accept the view that infection so seriously damages the pancreas as to impair its output of insulin.

It has been suggested that infection causes a stimulation of the thyroid and adrenal glands [cf. Lawrence and Buckley, 1927; Lawrence, 1927; Cramer, 1928; Evans and Zeckwer, 1927].

These last-mentioned authors investigated the hyperglycæmia produced in rabbits by the injection of killed *B. coli* and *B. proteus* cultures. Removal of one suprarenal gland, together with denervation of the other, completely prevented this type of hyperglycæmia, whilst ergotamine, administered simultaneously with the bacterial material, also abolished the usual hyperglycæmic response. Evans and Zeckwer, therefore, consider that bacterial hyperglycæmia is caused by central splanchnic stimulation, with resultant suprarenal overactivity, and increased glycogenolysis.

Still another suggestion advanced is that the products of the infection, such as toxins or trypsin (produced from pus or leucocytes), actually destroy or inactivate insulin [cf. Minkowski, 1926; Rosenthal and Behrendt, 1926; Karelitz, Cohen and Leader, 1930]. Although it is possible to demonstrate this inactivation *in vitro*, it is by no means certain that the same occurs in the body. In fact, there is definite evidence opposed to its occurrence [Lawrence, 1931].

Despite the number of investigations dealing with the nature of the diminished glucose tolerance occurring in infective states, the only chemical constituent of the blood that has been considered in detail is the glucose. The carbohydrate disturbance is probably more deeply seated, and in order to test this, it was decided to investigate the action of insulin on the liver glycogen of toxæmic young rabbits.

Goldblatt [1929] was the first to indicate the suitability of normal young rabbits for demonstrating the effect of small doses of insulin on liver glycogen. His observation that insulin caused a marked deposition of liver glycogen was confirmed under the same experimental conditions [Corkill, 1930]. Such a clearly defined action of insulin should be suitable for a study of the changes in carbohydrate metabolism produced by toxæmic states. Incidentally, such experiments might throw light on the mechanism by which insulin causes a deposition of liver glycogen in these young rabbits.

Goldblatt suggested that insulin acted merely by retarding the conversion of liver glycogen to glucose, with resultant hypoglycæmia and accumulation of glycogen. However, the fact that small doses of adrenaline were found to produce a similar deposition of liver glycogen, together with the evidence of Cannon, McIver and Bliss that insulin hypoglycæmia leads to a secretion of adrenaline, led me to suggest that the deposition of liver glycogen observed by Goldblatt probably involved the action of adrenaline, secreted in response to the insulin hypoglycæmia.

It is well known that a diphtheritic toxæmia produces marked degenerative changes in the suprarenal glands of laboratory animals. This fact suggested the need for investigating the effects of insulin and adrenaline in this toxæmia. Thus, if, in rabbits with definite suprarenal involvement, insulin failed to cause a deposition of liver glycogen, whereas insulin *plus* small doses of adrenaline did so, the rôle played by adrenaline would be clearly defined.

In addition to investigations dealing with this aspect of carbohydrate metabolism, comparisons have been made between the blood sugar

lowering effect of small doses of insulin, in normal and toxæmic young rabbits. The changes to be described present a clearly defined set of facts, which will be shown to support the conclusions of Evans and Zeckwer.

Experimental methods.

Young English hutch rabbits, bred at the Baker Institute, and from 6 to 8 weeks old, were used. Twenty-four hours before each experiment the young rabbits were placed in a cage without food. The determination of liver glycogen followed the procedure described in an earlier paper [Corkill, 1930] and, as in the previous experiments with insulin, the animals used were killed in a pre-convulsive stage of flaccidity and inco-ordination. When a number of blood-sugar determinations were carried out, blood was taken from the marginal ear vein, and glucose estimated according to the method of Hagedorn and Jensen. When only a single estimation was desired, blood was taken from the heart after the animal had been killed by a blow on the head. The diphtheria toxin was supplied by Mr Sutherland, of the Baker Institute, and the guinea-pig minimum lethal dose (M.L.D.) was 1/150th of a c.c. In the case of young rabbits 6 to 8 weeks old, 3·5–4 M.L.D. would usually cause death in 4–5 days. The stated quantity of toxin in each experiment was diluted to a volume of 4 c.c. with sterile normal saline and injected subcutaneously over the abdominal wall.

In previous experiments with normal young rabbits the quantity of insulin injected was 0·5 unit. In the present series of experiments it was found more suitable to use 1 unit as the initial dose, for both normal and toxæmic animals.

Results.

I. *The influence of insulin on the liver glycogen of young toxœmic rabbits.*

Experiment. In this first experiment an attempt was made to produce definite suprarenal damage and then to observe the effect of insulin on liver glycogen. Four young rabbits from the same litter were taken, and at the beginning of the experiment three of the animals had been without food for 24 hours. In addition, one of these latter animals was suffering from a toxæmia of 72 hours' duration induced by 4 M.L.D. of diphtheria toxin. One animal (No. 4), not at any stage deprived of food, was injected with 4 M.L.D. of diphtheria toxin and died 4 days after the injection. The remaining three animals were treated as is shown in Table I.

TABLE I.

No.	Rabbit wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1	820	1·50	0·034	Normal rabbit fasting 24 hours 9.20 a.m. 1 unit of insulin 11.25 „ Hypoglycæmic Killed
2	835	0·10	0·041	Toxæmia of 72 hours' duration Fasting 24 hours 10.30 a.m. 1 unit of insulin 2.15 p.m. 2 units of insulin 3.50 „ 2 „ 4.50 „ 2 „ 5.20 „ Inco-ordination Killed
3	824	0·30	0·093	Normal rabbit fasting 24 hours
4	—	—	—	Received 4 M.L.D. of diphtheria toxin Died 4 days later

The behaviour of the toxæmic rabbit No. 2 affords a clear contrast to that of the normal animal No. 1. Firstly, insulin appears less effective in producing a definitely hypoglycæmic state, and, secondly, there is a singular absence of the usual deposition of liver glycogen. Histological examination of the suprarenal glands of rabbit No. 2 revealed cloudy swelling of the cortical zones, together with slight medullary congestion. Sections stained by Kohn's formalin bichromate solution showed a marked diminution of adrenaline content, in comparison with similarly stained sections from the normal rabbits Nos. 1 and 3.

At this stage it was thought that these lesions would possibly provide a basis for further experiments with insulin and adrenaline. The fact that in the toxæmic animal No. 2 insulin appeared incapable of producing the usual deposition of liver glycogen, whilst, in addition, Kohn's staining revealed a marked diminution of adrenaline content, immediately suggested the necessity for investigating the action of insulin and adrenaline in such an animal.

Unfortunately the presence of another factor invalidated this apparently simple method of approaching the problem. Histological examination of the liver of rabbit No. 2 revealed the following changes: advanced degeneration of the central zones of the hepatic lobules with fragmentation, vacuolation and poor staining of liver cells, focal collections of lymphocytes and areas of actual necrosis.

Clearly, then, in view of this severe degree of hepatic damage, which would probably interfere with the storage capacity of the liver, one would not be justified in placing any direct significance on figures relating to glycogen storage.

This factor has not been fully realized in experiments similar to those carried out by Sweeney and Lackey [1928], where the glucose tolerance was studied, on successive days, in rabbits poisoned with diphtheria toxin. In experiments shortly to be described, it was found that the liver suffered from definite degenerative changes which progressed *pari passu* with the duration of the toxæmia.

Having due regard to the significance of hepatic damage in experiments of the nature just described, it was decided to select conditions where this was not a complicating factor. There still remained the possibility that, even in mild cases of toxæmia, some disturbance of carbohydrate metabolism might be present, and, accordingly, it was decided to investigate the action of insulin on liver glycogen in such states. In order to determine the most suitable conditions for experiments of this nature the following investigation was carried out.

Experiment. Five young rabbits from the same litter were taken and four were injected with 3·5 M.L.D. of diphtheria toxin. With the object of observing the development of toxic changes in the liver and suprarenal glands, the injected animals were killed at 24-hour intervals. Thus, the final rabbit presented the histological changes associated with a toxæmia of 96 hours' duration. In addition to the ordinary methods of histological examination, which were used for the liver and suprarenal glands, a small portion of each suprarenal was placed in Kohn's solution and examined for adrenaline content. Within 24 hours the liver may appear perfectly normal or show very slight toxic changes, chiefly about the central zones of the hepatic lobules. After 48 hours very definite degenerative changes are present, but they are not associated with any actual necrosis. From this stage onwards the degeneration becomes more marked, and definitely necrotic lesions may appear. The suprarenal glands afford, in one respect, a most interesting contrast to the lesions just described. Apart from some slight cloudy swelling of the cortical zones, no gross histological changes were observed at any stage by the ordinary methods of staining. When, however, the sections stained in Kohn's solution were examined, a pronounced contrast to the normal was found. Though not always marked at the 24-hour stage, there was from then onwards a definite diminution in the bichromate staining of the medullary cells.

In another series of experiments rabbits were again killed at 24-hour intervals after the injection of diphtheria toxin (3·5 M.L.D.), and extracts prepared from the suprarenal glands. These extracts were then tested by the spinal-cat blood-pressure method, and in this way the progressive effect of the toxæmia on the adrenaline content of the suprarenal glands

was studied. For the preparation of the suprarenal extract the glands were quickly removed and stripped clean of extraneous tissue. They were then ground up in a mortar containing sand and 3 c.c. of normal saline containing 2 p.c. acetic acid. Two further quantities of saline, each 2 c.c., were used to wash the extract into a test-tube, which was then placed in the boiling water bath for 5 min., the contents being then filtered. The quantities injected into the cat's femoral vein varied from 0·3–0·5 c.c. It was found that extracts prepared under identical conditions from normal rabbits of the same weight gave practically identical rises in blood-pressure. Hence, the tracings from these normal young rabbits could be used as a standard of comparison with toxæmic rabbits of the same age and weight.

Such experiments indicated that there was usually a slight but definite diminution of adrenaline content with a toxæmia of 24 hours' duration, and from this stage onwards the diminution became well marked and was reflected by the bichromate staining. Although in some cases there was no significant difference in the adrenaline content of extracts from normal rabbits and from animals suffering from a 24-hour toxæmia, in no instance did the suprarenal extract from a 24-hour toxæmia give a greater rise in blood-pressure than a similar extract prepared from a normal rabbit.

A study of the toxic changes occurring in the livers of these young rabbits would suggest that, using the above specified dose of toxin, one would be justified in studying glycogen storage in the liver when the toxæmia was of 24 hours' duration. At this stage slight or no histological changes are present, and it is not likely that the storage capacity for glycogen is interfered with. However, to make quite certain of this factor, it seemed necessary to estimate the liver glycogen of normal and toxæmic rabbits fed with the same amount of glucose.

Experiment. Four young rabbits from the same litter were taken and all food removed. In addition two animals were injected with 3 M.L.D.

TABLE II.

No.	Rabbit wt. g.	Liver glycogen p.c.	Remarks
1	610	0·20	Normal rabbit
2	620	1·50	Normal rabbit. Fed with 20 c.c. of 25 p.c. glucose. Killed 2 hours later
3	625	1·75	Toxæmic rabbit. Fed with 20 c.c. of 25 p.c. glucose. Killed 2 hours later. Liver showed slight cloudy swelling of central zones
4	615	1·60	Toxæmic rabbit. Fed with 20 c.c. of 25 p.c. glucose. Killed 2 hours later. Histologically the liver appeared normal

of diphtheria toxin. When the toxæmia was of 24 hours' duration the rabbits were treated as shown in Table II.

It cannot be suggested from the above figures that the toxæmia has in any way impaired the glycogen storage capacity of the liver. In addition half-hourly blood-sugar estimations were carried out on the rabbits Nos. 2 and 3, the results of which are shown below.

No. 2, normal rabbit	Blood sugar p.c.	No. 3, toxæmic rabbit	Blood sugar p.c.
Fasting	0·081	Fasting	0·079
½ hour after glucose	0·090	½ hour after glucose	0·113
1 hour ,,	0·125	1 hour ,,	0·146
1½ hours ,,	0·117	1½ hours ,,	0·150
2 hours ,,	0·108	2 hours ,,	0·143

Compared with the normal rabbit, No. 3 shows some degree of diminished glucose tolerance.

The experiments which are now to be described deal with the action of insulin in mildly toxæmic rabbits, that is to say, in young rabbits suffering from a toxæmia of 24 hours' duration induced by 3 M.L.D. of diphtheria toxin. Although several such experiments have been carried out, only a few results are included. The findings were always in the same direction, and afford a clearly cut conception of the changes induced by the toxæmia.

Experiment. Four young rabbits from the same litter were taken and kept without food for 24 hours. Two were injected with 3 M.L.D. of diphtheria toxin at the commencement of the fasting period.

The normal rabbit No. 3 shows the usual response to insulin, namely, a marked increase in liver glycogen as compared with the normal fasting control rabbit No. 2. Rabbit No. 4, which represents the effect of insulin in the presence of a toxæmic state, shows a marked contrast to the normal rabbit No. 3. Firstly, there is a singular absence of the usual deposition of liver glycogen, which, in view of what has already been discussed, cannot be attributed to defective storage power as a result of the toxæmia. Secondly, it would appear that much larger doses of insulin are required to provoke a definitely hypoglycæmic state, so that in this sense this animal shows an "insulin resistance." These two departures from the normal action of insulin in young rabbits have been confirmed in six experiments similar to that shown in Table III, and, although the resistance to the effect of insulin on the blood sugar was in some cases more pronounced than in others, in all such experiments I consistently

TABLE III.

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1	575	20·65	0·45	0·097	Toxæmic control Received 3 M.L.D. of diphtheria toxin
2	600	25·60	0·30	0·084	Normal control
3	620	30·00	2·6	0·034	Normal rabbit Received 1 unit of insulin Killed 2 hr. 20 min. later
4	595	25·15	0·15	0·045	Toxæmic rabbit 10 a.m. 1 unit of insulin 12.30 p.m. 1 ,, 2.30 ,, 2 units of insulin 4.0 ,, 2 ,, 5.30 ,, Hypoglycæmic Killed

failed to observe a deposition of liver glycogen. These observations gain an added interest in view of certain experiments which were published by Goldblatt [1930] while the present investigation was in progress. In this paper the author demonstrated that insulin could produce a marked deposition of liver glycogen in young kittens which were starved for 48 hours. In one experiment, instead of the usual deposition of liver glycogen, Goldblatt noted a considerable fall in the liver glycogen of the two kittens which received insulin. This apparently anomalous effect of insulin was attributed to the fact that these animals were suffering from a purulent infection of the eyes and "very probably had a high temperature." Beyond remarking that the severe infection had produced a response analogous to that found in rats, Goldblatt did not proceed further with this aspect of the problem. His observations would suggest that, as I have found insulin incapable of causing a deposition of liver glycogen in young rabbits suffering from a diphtheritic toxæmia, the same phenomenon may probably occur with other forms of bacterial toxæmia. The interesting fact that emerges from the experiments herein recorded is that the diphtheritic toxæmia has, indeed, converted the normal rabbit response to that of the normal mouse or rat.

In the following experiment the resistance to insulin was not so marked as that shown in Table III; nevertheless, insulin failed to cause a deposition of liver glycogen.

Experiment. Four young rabbits from the same litter were taken and three of them kept without food for 24 hours. In addition, two of these three were injected with 3 M.L.D. of diphtheria toxin at the time when the food was withdrawn. One animal (No. 4), not at any stage deprived of food, died 5 days after the injection of 3 M.L.D. of diphtheria toxin.

TABLE IV.

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1	800	23	0·30	0·107	Normal rabbit
2	820	25	2·1	0·040	Normal rabbit 1 unit of insulin Killed 3 hours later
3	745	22	0·21	0·038	Toxæmic rabbit 9 a.m. 1 unit of insulin 12 noon. 1 unit of insulin 1.30 p.m. Hypoglycæmic Killed
4	760	—	—	—	Received 3 M.L.D. of diphtheria toxin Food not withdrawn Died 5th day of toxæmia

In experiments similar to those just shown in Tables III and IV the toxæmic animals showed some degree of insulin resistance, and it might be thought that this phenomenon was in some way associated with the failure of these animals to show a deposition of liver glycogen. Further experiments, however, gave evidence directly opposed to such a theory. In one experiment, the results of which are shown in Table V, both the normal and toxæmic rabbits developed hypoglycæmic symptoms within approximately 2 hours after the injection of 1 unit of insulin. Nevertheless, a deposition of liver glycogen was not observed in the toxæmic rabbit.

Experiment. Four young rabbits from the same litter were taken and all food removed. In addition, two animals were injected with 3 M.L.D. of toxin. After 24 hours had elapsed the animals were treated as in Table V.

TABLE V.

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1	600	22·00	0·45	0·109	Normal control
2	580	20·15	0·37	0·098	Toxæmic rabbit
3	700	21·63	1·95	0·035	Normal rabbit 9.30 a.m. 1 unit of insulin 11.45 a.m. Hypoglycæmic Killed
4	640	20·34	0·32	0·042	Toxæmic rabbit 9.20 a.m. 1 unit of insulin 11.30 a.m. Hypoglycæmic Killed

The toxæmic rabbit No. 4, although otherwise comparable in its response to insulin with the normal animal No. 3, shows a marked dis-

crepancy in respect of liver glycogen. Although variations in the degree of insulin resistance were encountered, it could generally be predicted that toxæmic members of the same litter would show the same type of response.

Lawrence and Buckley, in their experiments with diphtheria-intoxicated adult rabbits, observed that the prelethal rise of the blood sugar could be abolished by ergotoxine and assessed this fact as further evidence in favour of their theory of thyreo-adrenal overactivity. Such evidence is in a sense related to the observations of Burn [1923], who showed that a small dose of insulin, administered to a well-fed rabbit, was much more effective in producing a hypoglycæmic state if the animal had previously received an intravenous injection of ergotoxine (5 mg.). Accordingly, it seemed worth while to see whether ergotoxine had any influence on the insulin resistance observed in these young toxæmic rabbits.

Experiment. Two groups of four rabbits were selected from two separate litters. The procedure adopted with each group was identical and as follows:

Two animals of each litter were injected with 3 M.L.D. of diphtheria toxin and all were kept fasting for 24 hours. One normal rabbit of each litter then received 1 unit of insulin hypodermically, whilst the two toxæmic ones were used to demonstrate the effect of insulin with and without a previous intravenous injection of 2·5 mg. of ergotoxine (Burroughs-Wellcome).

The combined results from the two litters are shown in Table VI.

Here, again, the toxæmic animals Nos. 5 and 6 show, in comparison with the normal ones Nos. 3 and 4, a definite resistance to insulin and no obvious glycogen storage. The rabbits which received ergotoxine before the insulin injection do not show any insulin resistance, but in spite of this, glycogen storage has not occurred. The above observations have been confirmed in two experiments similar to that shown in Table VI, and although in all instances ergotoxine appeared to annul the resistance to insulin, the values for liver glycogen were similar to those just described.

After the injection of the above specified dose of insulin normal young rabbits usually develop hypoglycæmic symptoms within 2–3 hours. On two occasions a delayed response was encountered in normal rabbits, so that these animals were comparable to some of the toxæmic ones, which showed only a mild degree of insulin resistance. However, the similarity ended here, since these normal rabbits, even though showing some degree of

TABLE VI. (The figures in brackets refer to the litter from which the animal was taken.)

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1 (L. 1)	720	23·45	0·28	0·110	Normal fasting control
2 (L. 2)	580	18·56	0·30	0·093	Normal fasting control
3 (L. 1)	750	24·20	2·3	0·028	Normal rabbit 9.30 a.m. 1 unit of insulin 11.45 ,, Inco-ordination Killed
4 (L. 2)	600	19·65	2·5	0·025	Normal rabbit 10.40 a.m. 1 unit of insulin 1.10 p.m. Hypoglycæmic Killed
5 (L. 1)	649	20·10	0·35	0·041	Toxæmic rabbit 10 a.m. 1 unit of insulin 12.30 p.m. 1 ,, 2.0 ,, 1 ,, 4.0 ,, 1 ,, 5.30 ,, Hypoglycæmic Killed
6 (L. 2)	715	20·00	0·41	0·035	Toxæmic rabbit 9.20 a.m. 1 unit of insulin 11.50 ,, 1 ,, 1.30 p.m. 1 ,, 3.0 ,, 1 ,, 4.30 ,, Hypoglycæmic Killed
7 (L. 1)	660	18·95	0·22	0·020	Toxæmic rabbit 10.5 a.m. 0·25 mg. ergotoxine intravenously 11.5 ,, 1 unit of insulin 1.20 p.m. Hypoglycæmic Killed
8 (L. 2)	610	20·10	0·11	0·032	Toxæmic rabbit 10.15 a.m. 0·25 mg. ergotoxine intravenously 11.15 ,, 1 unit of insulin 12.20 p.m. Hypoglycaemic Killed

resistance in comparison with the behaviour of most normal rabbits, had a definite deposition of liver glycogen, whereas the toxæmic animals always failed to show this response to insulin. The following experiment will illustrate this point, since it so happened that the normal and toxæmic animals corresponded very closely in their hypoglycæmic reaction to insulin.

Experiment. Four young rabbits were taken from the same litter and kept without food for 24 hours. Two received 3 M.L.D. of diphtheria toxin when the food was withdrawn.

The facts brought out in the preceding experiments would tend to invalidate any suggestion that the insulin resistance observed in the

toxæmic rabbits is in itself directly concerned with the failure of these animals to show glycogen storage. Thus, in Table V, the toxæmic animal showed no resistance to insulin, whilst in Table VII the normal animal

TABLE VII.

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1	800	20	0·3	0·098	Normal young rabbit
2	720	20·1	0·25	0·084	Toxæmic young rabbit
3	760	20	2·3	0·035	Normal young rabbit 9.30 a.m. 1 unit of insulin 11.30 „ 1 „ 1.30 p.m. 1 „ 3.0 „ Hypoglycæmic Killed
4	740	20	0·40	0·025	Toxæmic young rabbit 9.15 a.m. 1 unit of insulin 11.15 „ 1 „ 1.15 p.m. 1 „ 3.30 „ Hypoglycæmic Killed

had a delayed hypoglycæmic response comparable at least to some of the toxæmic ones. Nevertheless, in both cases the toxæmic animals failed to show a deposition of liver glycogen. Finally, as shown in Table VI, ergotoxine, although capable of abolishing the insulin resistance, did not facilitate the laying down of liver glycogen.

II. *The effect of insulin on the blood sugar of normal and toxæmic young rabbits.*

The experiments described in the preceding section demonstrate that, in the presence of a toxæmic state, insulin not only appears incapable of producing the usual deposition of liver glycogen, but, in addition, seems less effective in provoking a definitely hypoglycæmic state. In the present section an attempt is made to elucidate the nature of the resistance caused by the toxæmia to the effect of insulin on the blood sugar.

The results obtained differ in a marked fashion from those described by Lawrence and Buckley, who worked with adult toxæmic rabbits. I have found no evidence to suggest that, in the early stage of toxæmia, which is here under discussion, the fasting blood-sugar level is raised above the normal range. The fasting values found in both normal and toxæmic animals have shown low and remarkably constant figures. In addition, the fall in the fasting blood-sugar level produced by 1 unit of insulin,

within approximately 60 min. from injection, was practically the same in normal and toxæmic animals, although the subsequent behaviour of the blood sugar differed in the two groups. In all such experiments the animals fasted for 24 hours; it is not clear that this preliminary fast was adopted in all cases by Lawrence and Buckley. In their experiments on the effect of insulin given without diphtheria toxin, food had been withheld for from 17 to 20 hours, but in subsequent experiments, when diphtheria toxin had been injected, the only dietary control observed was based on the fact that these animals showed a disinclination for food, and ate very little as the toxæmia progressed. It is quite probable that this procedure may to some extent account for the differences in our results.

After the injection of 1 unit of insulin into normal or toxæmic young rabbits, the blood sugar fell usually within 60 min. to approximately 0·06 p.c., and then, in normal animals, more slowly to a hypoglycæmic level, at which definite symptoms occurred. In the case of toxæmic animals, instead of continuing to fall to this level, the blood sugar usually tended to rise again to its original fasting value, or even slightly higher. At this stage, in those animals where the insulin resistance was not marked, the injection of another unit of insulin would cause the blood sugar to fall to a hypoglycæmic level in a perfectly normal manner. In instances where the more marked degree of resistance was present, the blood sugar, in response to repeated injections of insulin, showed several cycles, in each of which the blood sugar first fell to about 0·06 p.c. and then returned to its original fasting level. Finally, after several injections, 1 unit of insulin produced a steady lowering of the blood sugar to a definitely hypoglycæmic level. In experiments similar to those shown in Table VI, where ergotoxine was injected prior to the administration of 1 unit of insulin, the blood sugar did not show the type of response that has been described above, but steadily progressed to a hypoglycæmic level; so that these toxæmic animals, in respect of the blood-sugar response, differed in no way from the normal rabbits which were injected with the same amount of insulin.

The following experiment, together with a graphical representation of the results (see Fig. 1), will illustrate the foregoing remarks. Although, in this experiment, the insulin resistance was not great (curve *A*), the essential nature of the blood-sugar changes is clearly indicated.

Experiment. Four young rabbits from the same litter were taken and all food removed from the cages. Two were injected with 3 M.L.D. of diphtheria toxin at the commencement of the fasting period, and

after 24 hours had elapsed the animals were dealt with as is shown in Table VIII.

TABLE VIII.

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Blood sugar p.c.	Remarks
1	670	21·0	1·80	See Curve *B*, Fig. 1	Normal rabbit 9.20 a.m. Fasting blood sugar =0·101 9.25 „ 1 unit of insulin 10.25 „ Blood sugar=0·064 11.25 „ „ =0·057 12.10 p.m. „ =0·035 Hypoglycæmic Killed
2	640	20·43	0·51	0·110	Normal rabbit
3	680	22·35	0·34	0·084	Toxæmic rabbit
4	660	21·24	0·28	See Curve *A*, Fig. 1	Toxæmic rabbit 10.45 a.m. Blood sugar=0·102 10.50 „ 1 unit of insulin 11.20 „ Blood sugar=0·074 11.50 „ „ =0·057 12.20 p.m. „ =0·061 12.50 „ =0·068 1.20 „ „ =0·093 1.23 „ 1 unit of insulin 2.20 „ Blood sugar=0·060 3.20 „ „ =0·052 3.50 „ „ =0·300 Hypoglycæmic Killed

The interpretation of these results is facilitated when they are considered in relation to certain other experimental data. In the experiment of Burn, previously referred to, a well-fed adult rabbit was taken and the blood-sugar response to 8 mg. of an early insulin preparation determined on two separate occasions. The first experiment was with insulin alone, whilst in the second, 5 mg. of ergotoxine were administered 2 hours prior to the insulin. With insulin alone the blood-sugar curve described by Burn was very similar to curve *A* in Fig. 1, which represents the changes in the blood sugar of a toxæmic young rabbit injected with 1 unit of insulin. In both animals insulin produced, within about an hour, a lowering of the blood-sugar level to approximately 0·060 p.c., but then, instead of progressing to a more definitely hypoglycæmic level, the blood sugar slowly rose to its original level, so that, within 2 hours, the insulin effect had practically disappeared. When ergotoxine was injected prior to the insulin, Burn found that the latter acted more effectively, so that now the blood-sugar curve was similar to that shown as *B* in Fig. 1.

In discussing his results, Burn has quoted the observations of McCormick, Macleod and colleagues [1923], from which it would appear that, whilst the initial fall of blood sugar caused by insulin is independent of the amount of liver glycogen, the rate at which the blood sugar returns to its normal level is largely governed by the amount of sugar

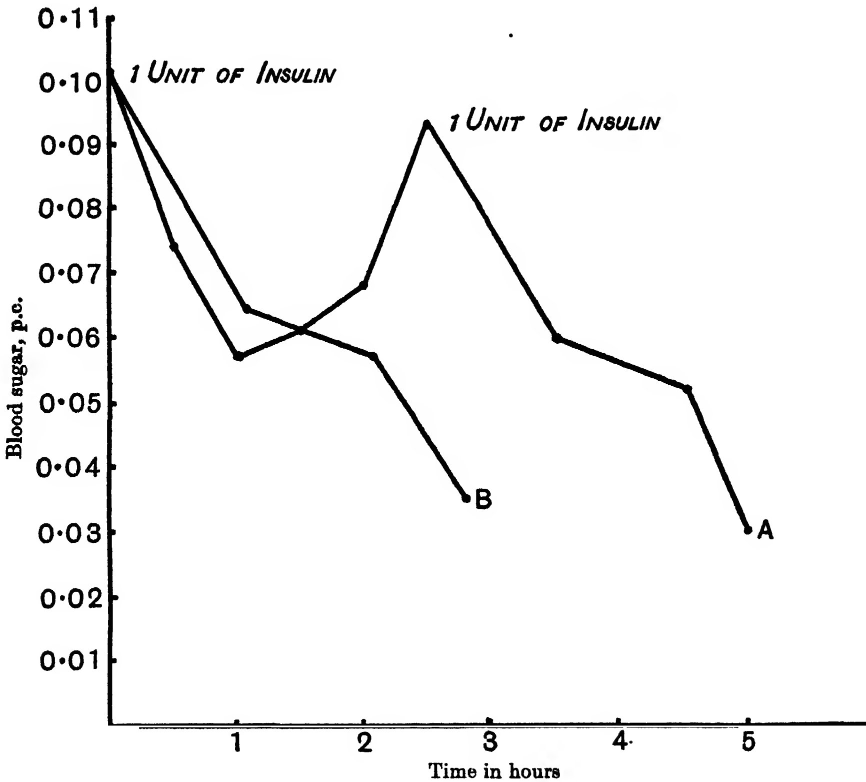

Fig. 1.

which the liver can supply. It is certainly a matter of common observation that adult well-fed rabbits, in comparison with animals that have fasted, require larger doses of insulin to produce hypoglycæmic symptoms. However, there is a fundamental difference between such well-fed animals and the young fasting rabbits that have been used in the present investigations. In the former class there is undoubtedly an abundant supply of liver glycogen, whilst in the latter, as judged by the control figures, only small quantities are likely to be present; so that in these animals a

high liver glycogen could not be the factor that determined the nature of their reaction to insulin.

It is now generally accepted that the hyperglycæmia produced by adrenaline is due to its action on a sympathetic mechanism in the liver by means of which glycogen is mobilized to glucose. The work of Cannon, McIver and Bliss indicates that adrenaline is liberated in response to insulin hypoglycæmia, so that these authors regard this phenomenon as a mechanism for protecting the body from dangerous hypoglycæmia. It would, therefore, appear reasonable to assume that, in the case of the adult well-fed rabbit, when the blood sugar had fallen to 0·06 p.c., the adrenaline secreted at this stage was able to mobilize sufficient glycogen as fully to compensate for the hypoglycæmic action of insulin. Ergotoxine is known to inhibit this sympathetic mechanism in the liver, so that insulin then acts without the complicating factor of glycogen mobilization; and under these conditions the blood sugar steadily progresses to a hypoglycæmic level.

The similarity in the blood-sugar curves of the young toxæmic rabbit and the adult well-fed rabbit, together with the effect of ergotoxine on these curves, suggests that, in both instances, we are dealing with a phenomenon dependent upon glycogen mobilization under the influence of the sympathetic nervous system, and at present we believe that adrenaline is the hormone chiefly concerned with the activation of this mechanism.

The evidence obtained from the preceding experiments, unlike that of Karelitz and his co-workers, does not suggest that the toxæmia has produced substances that directly inhibit the action of insulin. Thus, the initial fall of the blood sugar in response to 1 unit of insulin is similar in both normal and toxæmic animals and the reduced effectiveness of insulin in the well-fed adult rabbit and the young fasting toxæmic one would appear, as judged both from the blood-sugar curves shown in Fig. 1, and from the action of ergotoxine, to be dependent upon variations in the normal process of glycogen mobilization. The following somewhat isolated experiment with the spinal eviscerated preparation lends additional support to this view, since it demonstrates that a small dose of insulin, acting in the presence of a toxæmic state, is still effective in lowering the blood sugar. Adopting the technical procedures already described in a previous paper [Corkill and Marks, 1930], an attempt was first made to find a dose of insulin that produced only a moderate lowering of the blood sugar and then the effect of the same dose was studied in the presence of a diphtheritic toxæmia. It was found that 5–6 M.L.D. of diphtheria toxin usually caused the death of a cat weighing

about 3 kg. within 4–5 days, so that in a sense this degree of toxæmia is comparable to that used in the previous experiments.

Experiment. Insulin only. Cat weight = 2·5 kg. Spinal eviscerated preparation with suprarenal glands removed. Infusion of 4 p.c. glucose at the rate of 384 mg. an hour.

Time	1·0	1·30	2·10		2·40	3·10	3·40
Blood sugar, p.c.	0·223	0·218	0·218	↑ Insulin 5 units	0·195	0·150	0·132

Fall in blood sugar = 86 mg.

Experiment. Insulin in the presence of a toxæmia of 24 hours' duration. Cat weight = 2·8 kg. Spinal cord cut. Eviscerated and suprarenal glands removed. Infusion of 4 p.c. glucose at rate of 384 mg. an hour.

Time	12·45	1·10	1·30		2·0	2·30	3·0
Blood sugar, p.c.	0·262	0·250	0·247	↑ Insulin 5 units	0·228	0·189	0·159

Fall in blood sugar = 88 mg.

It cannot be suggested that the toxæmia has in any way interfered with the effect of insulin, as judged from that produced in the control animal. These results would naturally become more significant if it could be shown that the same degree of toxæmia was capable of producing a resistance to insulin in the intact cat. Owing to the difficulties in handling the cat for such an experiment, this was not done. Nevertheless, the facts appear to indicate that under these conditions there is no *in vivo* inactivation of insulin.

Note. The fasting blood-sugar values of the normal young rabbits used in the preceding experiments show much lower levels than those observed in previous investigations which were carried out in England. Dr Broben, who is in charge of the insulin standardization department at the Commonwealth Serum Laboratories, has furnished me with a series of 2000 fasting blood-sugar figures on adult rabbits, and the results are of the same order as those described in connection with the young rabbits used in the present investigation.

III. *Experiments with adrenaline.*

In attempting to explain the blood-sugar changes encountered in the toxæmic young rabbits, there are two possibilities to be considered, namely, that as a result of the toxæmia these animals are either more

sensitive to glycogenolytic agents, such as adrenaline, or respond more readily to hypoglycæmia, with an outpouring of adrenaline. Lawrence and Buckley were of the opinion that the former condition was present in their adult toxæmic rabbits, but it is not clear that their evidence warrants this assumption. In one of their experiments 0·5 mg. of adrenaline was injected into a rabbit when the toxæmia was of 5 days' duration, and the blood sugar rose from 0·120 to 0·305 p.c. within 1¾ hours. It was assumed from this single observation that "in the metabolic condition produced by the toxin, glycogenolytic agents cause an increased action." Apart from the fact that the dose of adrenaline was in itself rather in excess of that required to produce a definite hyperglycæmia (usually 0·2–0·25 mg. should prove ample for an adult rabbit), they have not compared the effect observed in the toxæmic animal with that produced by the same dose of adrenaline in a normal rabbit.

The question whether toxæmia increases the hyperglycæmic response to a given dose of adrenaline was tested by the experiments of which the results are given in Table IX.

TABLE IX.

No.	Rabbit wt. g.	1st test		2nd test (4 days later)
1*	800	Fasting blood sugar	=0·088	0·090
		0·14 mg. adrenaline		
		1 hour later blood sugar	=0·177	0·165
		2 hours ,,	=0·180	0·190
		Before the injection of toxin		In the presence of a 24 hour toxæmia
2	740	Fasting blood sugar	=0·090	0·086
		0·14 mg. adrenaline		
		1 hour later blood sugar	=0·168	0·110
		2 hours ,,	=0·176	0·090
3	780	Fasting blood sugar	=0·089	0·095
		0·14 mg. adrenaline		0·25 mg. adrenaline
		1 hour later blood sugar	=0·174	0·134
		2 hours ,,	=0·178	0·160

* This animal received no toxin.

The responses of two rabbits to 0·14 mg. of adrenaline were measured while still normal, and, 4 days later, in early diphtheritic toxæmia. A third animal, to control the effect of successive injections, received the two doses of adrenaline at the same interval, but no diphtheria toxin. It will be seen that the toxæmia has not increased the hyperglycæmic response; it has rather weakened it. If, therefore, the resistance to insulin hypoglycæmia is due to an augmented antagonism of the insulin effect by adrenaline, this increase must be due to a readier or more abundant output of adrenaline in response to the initial hypoglycæmia.

This conclusion is confirmed by the results of experiments shown in Table X, which were made to determine the effect of the toxæmia on the storage of glycogen in the liver, which a small dose of adrenaline, like a suitable dose of insulin, normally causes in the young rabbit.

TABLE X.

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Remarks
1	715	20	2·4	Normal rabbit Fasting blood sugar =0·104 0·14 mg. adrenaline 1 hour later blood sugar =0·179 2 hours ,, =0·190 Killed
2	680	19	0·48	Toxæmic rabbit Fasting blood sugar =0·083 0·14 mg. adrenaline 1 hour later blood sugar =0·132 2 hours ,, =0·170 Killed
3	693	21·3	0·53	Toxæmic rabbit Fasting blood sugar =0·110 0·14 mg. adrenaline 1 hour later blood sugar =0·141 2 hours ,, =0·152 Killed
4	720	20·15	0·35	Normal control

It will be seen that the toxæmia, while somewhat depressing the hyperglycæmia caused by adrenaline, suppresses the storage of liver glycogen in response to that substance, almost as completely as it suppresses the similar effect of insulin. It should be noted that, in order to obtain this effect of adrenaline on the liver of the normal animal, it is necessary to choose the dose sufficiently low to avoid a pronounced hyperglycæmia. The effect of adrenaline is not simple, and a dose high enough, in relation to the sensitiveness of the rabbit, to cause a large hyperglycæmia may cause loss rather than gain of glycogen by the liver. In the comparative experiments in Table X, however, there is no question of this. The hyperglycæmia in the toxæmic animals is milder than that in the normal animal, which shows a large increase of liver glycogen.

Since neither insulin nor adrenaline by itself, in doses causing accumulation of glycogen in the liver of the normal fasting young rabbit, produces this effect in a similar rabbit poisoned with diphtheria toxin, it was desirable to ascertain what the two hormones would do in this direction when injected together. Goldblatt [1929] showed that insulin and adrenaline together, in a dose containing such excess of the latter

TABLE XI. (Two litters, each comprising five rabbits, were used and the figures in brackets refer to the litter from which the animal was taken.)

No.	Rabbit wt. g.	Liver wt. g.	Liver glycogen p.c.	Remarks
1 (L. 1)	700	19·90	0·40	Normal rabbit
2 (L. 2)	920	21·14	0·16	Normal rabbit
3 (L. 1)	720	21·20	1·50	Normal rabbit Fasting blood sugar = 0·080 *1 unit of insulin + 0·18 mg. adrenaline 1 hour later blood sugar = 0·152 2 hours ,, = 0·132 Killed
4 (L. 2)	905	22	1·25	Normal rabbit Fasting blood sugar = 0·110 1 unit of insulin + 0·18 mg. adrenaline 1 hour later blood sugar = 0·154 2 hours ,, = 0·132 Killed
5 (L. 1)	650	21·20	1·50	Normal rabbit 1 unit of insulin Killed 2 hours 20 min. later
6 (L. 2)	878	18·35	1·80	Normal rabbit 1 unit of insulin Killed 2 hours later
7 (L. 1)	930	19·40	0·17	Toxæmic rabbit Fasting blood sugar = 0·108 1 unit of insulin + 0·18 mg. adrenaline 1 hour later blood sugar = 0·115 2 hours ,, = 0·065
8 (L. 1)	950	20·1	0·18	Toxæmic rabbit Fasting blood sugar = 0·106 1 unit of insulin + 0·18 mg. adrenaline 1 hour later blood sugar = 0·124 2 hours ,, = 0·097
9 (L. 2)	680	20·0	0·10	Toxæmic rabbit Fasting blood sugar = 0·083 1 unit of insulin + 0·18 mg. adrenaline 1 hour later blood sugar = 0·075 2 hours ,, = 0·061
10 (L. 2)	700	20·25	0·15	Toxæmic rabbit Fasting blood sugar = 0·099 1 unit of insulin + 0·18 mg. adrenaline 1 hour later blood sugar = 0·079 2 hours ,, = 0·061

* The insulin and adrenaline were not mixed but injected separately into the subcutaneous tissues of opposite thighs. The appropriate quantity of 1/10,000 adrenaline was made up to a volume of 2 c.c. with normal saline.

that the effect, on balance, was a moderate increase of blood sugar, caused a deposition of glycogen in the liver similar to that produced by the same dose of insulin alone. My own experiments with the combined effect on normal rabbits, as shown in Table XI, give a very similar result. In spite of the fact that a moderate rise of blood sugar is produced, in

place of the fall caused by the same dose of insulin alone, the gain of liver glycogen is not significantly different, in the normal rabbits receiving the combined treatment, from that seen in those receiving insulin by itself.

In the toxæmic rabbits, on the other hand, although the hyperglycæmia is slight, or replaced by a mild degree of hypoglycæmia, the treatment with insulin and adrenaline together is just as ineffective, in causing the deposition of glycogen in the liver, as was that with either hormone separately.

Discussion.

In my earlier paper I gave reasons for believing that, when either insulin or adrenaline is injected into a normal animal, the effect produced, and especially that on the liver glycogen, is not the result of an uncomplicated action of the hormone injected, but involves the intervention of a reactive increase in the output of the other, from the suprarenal medulla or the pancreatic islets as the case may be. I suggested further, but without the support of experimental evidence, that other hormones might also participate in what seemed to be a complex effect. The results above recorded certainly do not weaken the impression of the complexity of the problem, and of the difficulty of framing a comprehensive interpretation of all the data.

If the effects of diphtheria poisoning on the response of the blood sugar to insulin stood by themselves, they would present no special difficulty. In the early period after injection of insulin, the blood sugar shows, in the poisoned animal, a steep decline very similar to that seen in the normal. The difference in final effect is due to an early interruption of the fall by a secondary rise, which we can reasonably attribute to an excess of the normal effect of a reactive output of adrenaline, in promoting recovery from insulin hypoglycæmia. This supposition is supported by the fact that ergotoxine removes this abnormally early recovery. Since adrenaline, artificially injected, produces a rather less severe hyperglycæmia in the poisoned animal than an equal dose produces in the normal animal, the early recovery is not due to abnormal sensitiveness to this effect of adrenaline. It must, therefore, be due to an abnormally ready, or abnormally large, output of adrenaline from the glands; and this is in accordance with the observation that the suprarenal medulla does, indeed, lose its adrenaline with abnormal rapidity in these toxæmic animals. So far the facts appear to fit a relatively simple conception.

The effects of the diphtheria poison on the accumulation of liver glycogen are much more difficult to interpret. The normal effects of insulin or adrenaline alone, in causing such accumulation, are suppressed. If the deposition really required a balanced action of the two hormones, and if we rightly interpret the effects on the blood sugar as indicating an over-ready response of the suprarenal gland to hypoglycæmia, we should expect insulin to promote the deposition of liver glycogen with unusual readiness in the toxæmic animal. Similarly adrenaline, causing less hyperglycæmia in the toxæmic than in the normal animal, might be expected to cause the deposition of glycogen the more easily. In both cases the effect observed is the opposite of that expected. Again, if we suppose that the toxæmia upsets, in either direction, the response of the endocrine organ to the effect of the alternative hormone, we ought to be able to restore the balance by giving insulin and adrenaline together, in proportions which cause glycogen deposition in the liver of the normal animal; but again the effect is suppressed by the toxæmia. The interpretation, again, would be simple if we could suppose that the toxin had deprived the liver cells of their power of forming and holding glycogen. The dose of toxin and the time of its action were so chosen, however, as to ensure that the liver was still capable of storing glycogen normally when glucose was administered.

The only safe conclusion, for the present, appears to be that the effect of the toxin, at a stage when the liver can still store glycogen, interrupts at some point the complex chain of events, which leads to such storage when an injection of insulin or adrenaline, or of both together, is made into a normal young rabbit. The complexity may be illustrated by considering the associated increase of both liver glycogen and blood sugar when a small dose of adrenaline is given to a normal young rabbit. The rise of blood sugar may be attributed to the accelerated conversion into glucose of glycogen already existing in the liver; but this must be more than counterbalanced by the new formation of liver glycogen from some other substance than glucose. Some of it is known to be formed from lactic acid, liberated by accelerated breakdown of muscle glycogen. To determine, however, whether enough glycogen is lost from the muscles, to account for the observed increase in the liver as well as for what is again shed into the blood as glucose, we should require detailed analyses, including determinations of respiratory exchange, on rabbits in sufficient numbers to eliminate the errors due to individual differences. Without such data as would enable the contribution of each factor of the complex to be assessed, it is impossible to identify the particular defect in the

rabbit with early diphtheria toxæmia. We only know that the defect is such as to prevent the normal increase of liver glycogen, when either insulin or adrenaline is injected, with relatively small and inconstant disturbance of the effect on the blood sugar. The knowledge emphasizes the complexity of the effect on the carbohydrate balance, produced when either hormone is injected into the normal animal; and it again shows clearly that there is no direct causal relationship between accumulation of liver glycogen and fall of blood sugar under the action of insulin.

Summary.

1. The actions of insulin and adrenaline have been studied in normal young rabbits, and in similar rabbits at an early stage of poisoning with diphtheria toxin.

2. Although both these hormones cause a deposition of liver glycogen in young fasting rabbits, neither can produce this effect in the toxæmic animals, whether given separately or in combination.

3. In most of the toxæmic animals a resistance to the effect of insulin on blood sugar was observed, having the nature of early recovery from hypoglycæmia.

4. Ergotoxine, although abolishing this insulin resistance, does not facilitate the deposition of liver glycogen.

5. The bearing of these observations on the complex action of insulin in the normal animal is discussed.

I am greatly indebted to Sir Henry Dale and to Dr Penfold for much helpful advice and criticism, and also to Dr Willis for his continuous assistance in the histological examinations that were necessary in the present investigations.

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THE EFFECTS OF THE COMPONENTS OF LECITHINE UPON DEPOSITION OF FAT IN THE LIVER.

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The results of the experiments in which purified lecithine from two sources, egg yolk and fresh beef liver, has been shown to prevent deposition of fat in the livers of normal rats [Best, Hershey and Huntsman, 1932] have led us to study the effects of feeding various components of lecithine. It appeared possible that the oleic or other unsaturated fatty acid, the glycerophosphate, the choline, some combination of two or more of these factors, or the whole lecithine molecule, might be the active agent. The general procedure in this series of experiments has been to substitute for the lecithine in the daily diet of each rat the amounts of glycerophosphate, oleate, or choline, which it is calculated might be derived from 0·5 g. of purified lecithine. This amount of lecithine was previously found to be effective in preventing the deposition of fat in the livers of normal white rats, each of which ingested daily for 3 weeks approximately 2·5 g. of fairly well-saturated fat.

Methods.

The methods used in this study were in general similar to those discussed in the preceding paper. Any slight change will be noted in the description of each experiment. The required amount of the material under test was dissolved in water, except in the case of sodium oleate, and thoroughly mixed each day with the diet, which contained approximately 40 p.c. fat (beef dripping).

Experimental results.

(1) *Glycerophosphate.* In the first experiment the effect of sodium β glycerophosphate was tested; 0·12 g. was added to the daily diet of each rat. The values obtained in the control experiment are given in Table I. The results for the animals which received sodium β glycerophosphate

TABLE I. Fat diet. Stock diet plus fat (fat 40 p.c. of total food).

Rat No.	Total fat eaten (g.)	Fat eaten per diem (g.)	Fat excreted per diem (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	p.c. fatty acids in liver	Iodine No.
41	52·5	2·5	0·13	152	158	12·8	82
42	52·5	2·5	0·16	174	174	23·5	80
43	52·5	2·5	0·15	182	184	9·5	87
44	52·5	2·5	0·17	188	186	21·9	90
45	52·5	2·5	0·15	186	184	7·6	88
46	52·5	2·5	0·19	200	194	22·3	86
47	52·5	2·5	0·17	214	204	7·8	84
48	52·5	2·5	0·16	212	212	20·0	96
49	52·5	2·5	0·19	264	244	15·4	96
50	52·5	2·5	0·17	232	222	15·7	99
						Average = 15·6	

are given in Table II. In another series of experiments in which calcium α glycerophosphate was used the average liver fatty acid of the control group was 17·3 p.c. and that of the test group 19·5 p.c. These results indicate that deposition of fat in the liver is not prevented by the glycero-

TABLE II. Fat diet plus sodium glycerophosphate. Stock diet plus fat (fat 40 p.c. of stock diet plus fat ration) plus sodium glycerophosphate.

Rat No.	Total fat eaten (g.)	Fat eaten per diem (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Sodium glycero-phosphate per diem (g.)	p.c. fatty acids in liver	Iodine No.
111	52·5	2·5	164	168	0·12	9·2	148
112	52·5	2·5	154	162	0·12	20·1	85
113	52·5	2·5	170	160	0·12	14·8	82
114	52·5	2·5	170	170	0·12	15·8	57
115	52·5	2·5	210	198	0·12	7·4	100
116	52·5	2·5	210	190	0·12	8·3	107
117	52·5	2·5	220	210	0·12	21·7	89
118	52·5	2·5	214	198	0·12	23·6	96
119	52·5	2·5	238	230	0·12	22·4	90
120	51·1	2·4	242	214	0·12	36·3	77
						Average = 18·1	

phosphoric acid derived from the lecithine. In some preliminary experiments performed several years ago, in which sodium phosphate was added to a diet high in fat, Hershey and Soskin obtained similar negative results.

(2) *Sodium oleate.* Approximately the amount of sodium oleate which might be formed as a result of the decomposition of 0·5 g. of purified lecithine was added to the daily ration of each test rat in this series (Table IV). The results for the controls of this series are given in Table III. These results demonstrate that sodium oleate does not inhibit fat deposition in the liver under the conditions of these experiments. In other

TABLE III. Fat diet. Stock diet plus fat (fat 40 p.c. of total food).

Rat No.	Length of exp. (days)	Total fat eaten (g.)	Fat eaten per diem (g.)	Fat excreted per diem (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	p.c. fatty acids in liver	Iodine No.
1	20	50·0	2·5	0·17	210	200	9·2	97
2	20	50·0	2·5	0·15	190	184	11·0	95
3	20	50·0	2·5	0·16	170	174	16·0	90
4	20	50·0	2·5	0·15	170	166	8·5	94
5	21	52·5	2·5	0·17	180	176	21·1	—
6	21	52·5	2·5	0·16	176	174	8·2	100
7	21	52·5	2·5	0·16	180	170	11·7	95
8	21	52·5	2·5	0·17	182	170	28·2	97
9	21	51·5	2·5	0·18	166	152	13·8	89
10	21	52·5	2·5	0·14	162	158	16·4	91
							Average = 14·4	

TABLE IV. Fat diet plus sodium oleate. Stock diet plus fat (fat 40 p.c. of stock diet and fat ration) plus sodium oleate.

Rat No.	Length of exp. (days)	Total fat eaten (g.)	Fat eaten per diem (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Sodium oleate per diem (g.)	p.c. fatty acids in liver	Iodine No.
61	20	50·0	2·5	200	210	0·19	7·5	111
62	20	50·0	2·5	190	200	0·19	9·2	114
63	21	51·5	2·5	164	162	0·19	14·2	74
64	21	50·5	2·4	180	180	0·18	17·4	94
65	21	50·2	2·4	180	188	0·18	13·6	93
66	21	52·5	2·5	205	208	0·19	18·2	95
67	21	52·5	2·5	166	170	0·19	14·0	99
68	21	52·5	2·5	162	174	0·19	9·2	95
69	21	52·5	2·5	178	182	0·19	6·8	104
							Average = 12·2	

experiments in which much larger amounts of sodium oleate were provided, negative results were also obtained.

(3) *Choline.* The effects of various amounts of choline have been studied. The largest amount given, 117 mg. daily, appeared to be fairly well tolerated. The smallest dose used (10 mg. daily) produced a definite effect on deposition of liver fat, but the average value in this series was

TABLE V. Fat diet plus choline. Summary of results.

Choline per day per rat (mg.)	No. of rats	Average fat eaten per diem (g.)	p.c. fatty acids in liver	Iodine No.
0	37	2·5	16·2	97
10	10	2·5	9·7	95
20	9	2·5	4·9	102
40	9	2·5	5·5	110
66	6	2·5	3·5	135
70	10	2·5	5·2	103
100	5	2·4	3·4	—
117	10	2·4	5·1	111

considerably higher than in other animals from the same colony which received larger amounts of choline. The average figures for the fatty acid content of the livers of all the control and test animals are given in Table V, while the results for the individual rats in one series are given in Table VI. The controls for this group are the same as those receiving

TABLE VI. Fat diet plus choline. Stock diet plus fat (fat 40 p.c. of stock diet and fat ration) plus choline.

Rat No.	Total fat eaten (g.)	Fat eaten per diem (g.)	Fat excreted per diem (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Choline per diem (g.)	p.c. fatty acids in liver	Iodine No.
51	52·5	2·5	0·19	210	206	0·07	3·8	112
52	51·8	2·5	0·15	170	168	0·07	5·6	98
53	43·8	2·1	0·17	170	164	0·06	6·1	98
54	52·5	2·5	0·15	178	158	0·07	5·4	91
55	52·5	2·5	0·16	182	180	0·07	6·2	100
56	52·5	2·5	0·12	190	190	0·07	3·2	126
57	52·5	2·5	0·15	210	210	0·07	5·6	106
58	52·5	2·5	0·16	212	198	0·07	3·9	109
59	52·5	2·5	0·16	242	218	0·07	8·0	88
60	52·5	2·5	0·23	240	222	0·07	4·2	104
						Average	=5·2	

sodium glycerophosphate and sodium oleate (Table I). Figures for daily fat excretion are given in Table I and Table VI. *In each of the seven experiments in which choline was administered, deposition of liver fat was unmistakably less than in the control animals.*

The effect of choline administered subcutaneously.

In two short series of experiments the effect of choline injected subcutaneously has been studied. In the first series 5 mg. administered in one dose daily produced no detectable effect upon the deposition of liver fat. In the second series 10 mg. were administered at each injection, and each test rat received for a period of 3 weeks two injections on Saturdays and Sundays, and four on other days. This treatment resulted in an average liver fat of 3·8 p.c. in the 10 test rats. The control animals of this series received the same volume of saline and the same number of injections as those to which the choline was administered. This treatment did not agree well with either the control or test animals, and they were not in good condition at the end of the experiment. The average liver fatty acid of the control animals was 7·4 p.c. Since a comparable group of animals receiving the same diet, but no injections of saline, had an average liver fatty acid content of 15·6 p.c. (Table I) it is apparent that the frequent handling and subcutaneous injections interfered with de-

position of liver fat. The choline chloride may have been slightly more irritating on subcutaneous injection than the saline, but the animals which received choline were in quite as good condition as those that had saline at the termination of the experiment. It appears, however, that choline administered subcutaneously does inhibit fat deposition in the liver under the conditions of this experiment. Another group of ten animals in this series received subcutaneously 0·5 c.c. each day of a suspension containing 50 mg. of purified beef liver lecithine in distilled water. The lecithine was not well absorbed, and lumps developed at the sites of the injections. The average liver fatty acid content at the end of 3 weeks was 5·2 p.c. (controls 7·4 p.c.). The results of these preliminary experiments suggest that the subcutaneous administration of the factor or factors which affect deposition of liver fat in the white rat is unlikely to be satisfactory.

Effect of some other substances.

Amino-ethyl alcohol. Since the purified lecithine which has been used contained a small amount of amino-nitrogen, the effect of amino-ethyl alcohol was studied. The results obtained in the test animals are given in Table VII, and those for the controls in Table III.

TABLE VII. Fat diet plus amino-ethyl alcohol. Stock diet plus fat (fat 40 p.c. of stock diet and fat ration) plus amino-ethyl alcohol.

Rat No.	Length of exp. (days)	Total fat eaten (g.)	Fat eaten per diem (g.)	Wt. or rat before (g.)	Wt. of rat after (g.)	Amino-ethyl alcohol per diem (g.)	p.c. fatty acid in liver	Iodine No.
51	20	47·5	2·4	170	167	0·036	11·0	107
52	20	50·0	2·5	196	208	0·038	10·7	104
53	21	52·5	2·5	198	190	0·038	12·7	95
54	21	52·5	2·5	185	175	0·038	10·6	92
55	21	52·5	2·5	170	172	0·038	14·4	87
56	21	51·0	2·4	164	160	0·037	16·7	90
57	21	52·5	2·5	164	170	0·038	14·8	97
58	21	52·5	2·5	194	—	0·038	6·2	86
							Average = 12·1	

Betaine. The activity of choline in preventing deposition of liver fat immediately focused our attention on substances of similar chemical constitution. A comprehensive study of related compounds is contemplated. It is very interesting that an apparently positive result has been obtained with betaine. The results for the betaine controls are given in Table I, and those of the test animals in Table VIII.

TABLE VIII. Fat diet plus betaine. Stock diet plus fat (fat 40 p.c. of stock diet and fat ration) plus betaine.

Rat No.	Total fat eaten (g.)	Fat eaten per diem (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Betaine per diem (g.)	p.c. fatty acids in liver	Iodine No.
101	52·5	2·5	160	164	0·12	5·6	70
102	52·5	2·5	158	168	0·12	10·7	79
103	49·1	2·3	170	170	0·11	6·1	116
104	52·5	2·5	180	178	0·12	3·6	114
105	51·1	2·4	190	182	0·12	4·6	120
106	52·5	2·5	200	200	0·12	5·5	98
107	52·5	2·5	212	202	0·12	3·8	146
108	52·5	2·5	216	206	0·12	7·8	96
109	51·6	2·5	212	202	0·12	5·0	109
110	52·5	2·5	234	226	0·12	5·8	137
						Average = 5·9	

Discussion.

The results of the experiments reported in this paper establish the fact that neither sodium nor calcium glycerophosphate nor sodium oleate, when fed daily in amounts which might be derived from 0·5 g. of lecithine, inhibits the deposition of fat which takes place in the livers of control white rats under the conditions of our experiments. Choline chloride, however, added to the stock diet inhibits in some way this accumulation of fat in the liver. The possibility that oleate or glycerophosphate may influence this action of choline has not yet been investigated.

The fact that amino-ethyl alcohol, when fed daily in amounts which might be derived from 0·5 g. of kephaline, does not inhibit deposition of fat in the livers of rats, suggests that kephaline plays no part in the "lecithine" effect. The apparently positive result obtained when betaine was added to the diet indicates that other compounds containing penta-valent nitrogen should be investigated.

$$(CH_3)_3{\equiv}N\begin{matrix}\diagup OH \\ \diagdown CH_2{-}CH_2OH\end{matrix} \qquad\qquad (CH_3)_3{\equiv}N\begin{matrix}\diagup OH \\ \diagdown CH_2{-}COOH\end{matrix}$$

Choline   Betaine

It may be necessary to determine the relative potencies of betaine and choline and perhaps of other compounds before proceeding with the study of the physiological significance of the factor or factors which modify deposition of liver fat.

In the previous paper [Best, Hershey and Huntsman, 1932] a number of very high iodine numbers were reported for the liver fatty acids of one group of animals which received crude liver lecithine. We have

encountered similar very high values in only one of the choline-fed series. While we have no ground, other than that of improbability in the light of present knowledge, for suspecting the reliability of these high iodine numbers, we wish to reserve judgment on their probable significance until a more adequate study of this aspect of the problem has been carried out. These very high values are not included in Table V. In addition to the great variation between animals from different colonies and individuals from the same colony, the somewhat irregular physiological activity of commercial choline preparations must be considered in attempting to locate the causes of aberrant results. The possibility that certain specimens of liver may contain substances which interfere with determinations of iodine numbers by Wijs' procedure merits further investigation.

Since we intend to follow several of the leads suggested by the results of the experiments reported above, it will be advisable to wait for further information on certain points before expressing an opinion on the physiological significance of this action of choline chloride. We are at present attempting to determine the action of choline in various species of small animals. The deposition of liver fat in mice is apparently influenced by choline in the same way as in rats. Experiments are also already well under way in which the effect of choline (1) on the lipæmia which may be produced in diabetic dogs and (2) on the condition in these animals characterized by fatty degeneration of the liver, which has been referred to in previous communications, may be studied. Until the action of choline on depancreatized dogs has been thoroughly investigated there is no direct evidence for the attractive assumption that the mechanisms of the "lecithine" effects in diabetic dogs and normal rats are essentially similar. The results of one experiment on a depancreatized dog suggest, however, that this may prove to be the case.

While it is obvious that the effects of choline or other active substances on fatty changes in the livers of experimental animals produced by various means (phosphorus or chloroform poisoning) may prove interesting fields of investigation, it is equally evident that much more information on normal animals can profitably be obtained before studies of that kind are initiated.

In interpreting the results of these experiments, the values obtained from the analyses of the livers for total fatty acid are the only ones upon which we have relied. It is interesting, however, that in an experiment in which adequate amounts of a substance under test have been provided, the results can always be predicted from the appearances of the livers of

the control and test animals. Livers containing large amounts of fatty acid are yellowish in colour, and those in which the fat content is extremely high are very friable. The livers from the test animals are approximately normal in colour, and are smaller and firmer than those from the controls.

SUMMARY.

The effects of the various constituents of lecithine upon the deposition of fat in the livers of normal white rats under the conditions described in the previous paper [Best, Hershey and Huntsman, 1932] have been determined. The results indicate that neither the unsaturated fatty acid (sodium oleate) nor the glycerophosphate (sodium or calcium glycerophosphate) is the active factor in the "lecithine" effect. Since our purified lecithine contained a small amount of amino-nitrogen, the action of amino-ethyl alcohol was also determined. Here again the results were negative. Choline (choline chloride), on the other hand, administered by mouth has consistently inhibited the deposition of fat in the livers of the rats under the conditions of our experiments. No evidence has been obtained that choline increases the excretion of fat. In one experiment positive results have been obtained with betaine. This finding indicates that further investigation of compounds containing pentavalent nitrogen may be profitable. A discussion of the possible physiological significance of these results is reserved until further experiments on rats and on other species of animals have been conducted.

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OBSERVATIONS ON EXTRACTS OF BEEF ADRENAL CORTEX AND ELASMOBRANCH INTERRENAL BODY.

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The recent development of methods for the preparation of extracts of the adrenal cortex, which are capable when injected into adrenalectomized cats of reviving them from the symptoms of adrenal insufficiency and of prolonging their lives [Hartman, Brownell and Hartman, 1930; Swingle and Pfiffner, 1931 *a*, *b*], renders possible a new approach in the investigation of functions of the cortex. With the aid of this advance two problems have been undertaken. The first, described in Part II of this paper, is an attempt to ascertain whether the structure in elasmobranch fish, known as the interrenal body and which is supposed to be the homologue of the mammalian adrenal cortex, contains the same active substance as is present in beef adrenal cortex. The second concerns the averred influence of the adrenal cortex on sex functions and development and will be dealt with in a subsequent paper.

Since a cortical extract of proved potency was necessary, and none was on the market when this work was begun, the preparation and testing of the material at first hand was imperative. Certain facts of interest emerging from this preliminary work justify its being dealt with more fully than would otherwise be necessary.

Part I.

(i) *The extract of beef adrenal cortex.*

In selecting one of the two available methods for preparing an active cortical extract, that of Swingle and Pfiffner [1931 *b*] was chosen because the published accounts of technique and experimental results were more satisfactory. In this procedure the minced, fresh, whole, beef glands are extracted several times with ethyl alcohol of which the concentration is maintained at about 80 p.c. by volume. The alcohol is removed from the filtered extract by distillation under reduced pressure at 40–50° C. The resulting residue is similarly treated in turn with benzene and with

acetone. The acetone-soluble residue is further purified by repeated dis tribution between 70 p.c. alcohol and petroleum ether of low boiling point. The alcohol fractions are further washed with petroleum ether, combined and all but a trace of adrenaline removed by allowing the alcohol solution to percolate through permutit. On removing the alcohol and adding water a part remains out of solution. The aqueous solution, 1 c.c. per 50 g. of original gland, is prepared for subcutaneous injection by passing through a sterile Seitz filter.

By this technique thirty-two batches of extract have been prepared, care being taken to use pure solvents. The following minor modifications in the routine work were found necessary.

(*a*) It was impracticable to arrange that the glands should be regularly placed on ice immediately on removal from the animals at the slaughter house. Consequently, in most instances, they had been at room temperature from 2–6 hours before being placed in alcohol.

(*b*) Unfortunately, the quantity of glands available on any day was small and, to maintain some degree of economy in their extraction, the alcoholic extracts of those obtained on a number of days had to be accumulated and combined. Consequently, the tissue first obtained was extracted in alcohol for 8–10 days and the last but 3-5 days.

(*c*) The water used in the final stage of later extracts was adjusted to *p*H 5 by addition of *N* HCl, since it was found that this facilitated Seitz filtration.

One batch of glands was treated by a modified process as follows: The minced tissue was extracted repeatedly with a mixture of benzene and alcohol in equal volumes, in the hope that the low boiling point of the ternary mixture would facilitate the subsequent removal of water under reduced pressure and render possible extraction of the tissue with an anhydrous organic solvent. For this latter purpose benzene was used repeatedly, in presence of large quantities of anhydrous sodium sulphate. The benzene washings were filtered off through a Büchner funnel, combined and distilled. Thence the residue was treated as in the Swingle and Pfiffner method.

No particular difficulty was encountered in the preparation of these extracts by the Swingle and Pfiffner method and only one noticeable departure from the described nature and behaviour of the material in the process of preparation was regularly observed. This concerns the final stage of the process. Seitz filtration of the watery extract is rapid according to Swingle and Pfiffner, and the *p*H of the finished product about 5. Our experience is to the contrary, Seitz filtration being slow

(1–1½ hours) and not always complete, and the *p*H of the final extract about 6·8 (quinhydrone electrode). From Dr Pfiffner [1932] we learn that the *p*H of the distilled water in Prof. Swingle's laboratory is between 6·2 and 6·6. The water used in this laboratory has a *p*H value in the region of 7. Adjusting the *p*H of the water used in this final stage made no difference in the rate of filtration until a *p*H of 5 was reached. It then became rapid. Extracts prepared from glands placed on ice within 20 min. of death of the animals had a *p*H value in no way different from that obtained from glands in the cooling of which there was long delay, nor was the *p*H different in extracts prepared from glands kept one night in the refrigerator. Further, repeated estimation of the lactic acid content of fresh gland tissue and of a sample from the same gland after 24 hours in the refrigerator showed no accumulation of lactic acid.

Decided alkalinity to which two extracts were subjected did not destroy their potency. In these instances alkali was acquired from permutit which had not been washed free of the NaOH used in cleansing it. After neutralizing the alkali, the administration of these extracts to a cat suffering a severe degree of adrenal insufficiency had a distinctly beneficial effect (see Fig. 1, Cat 6, Part II).

The adrenaline content of typical extracts, as judged by the effect on the blood-pressure of both the decerebrate and decapitate cat, showed a pressor effect equivalent to a concentration of 1 part in 1–2 million. No extract was found to give the biuret reaction.

The subcutaneous injection of 0·5 c.c. of extract of beef adrenal cortex into adult albino mice, of 0·1 c.c. into young, 6–8 g. mice, or of 10 c.c. intraperitoneally in cats produced no untoward results. The intraperitoneal injection of 1 c.c. extract into young 25 g. rats caused severe reactions in the few experiments in which this method of administration was tried. These reactions appeared within 1–2 min. of injection and were characterized by extreme prostration and rapid breathing. Recovery took place slowly over 2–4 hours and was always complete. Young mice receiving 0·1 c.c. intraperitoneally reacted similarly though not so severely.

Histamine was apparently the causative agent of these toxic symptoms as tests of the extract by Dale's method [Burn, 1928] seem to show. The intravenous injection of 0·3 c.c. of the extract into a cat under ether caused a slight rise in blood-pressure followed by a fall. After exposure of some extract to an equal amount of $2N$ NaOH for an hour, to destroy the adrenaline, and neutralization by $2N$ HCl, the injection of 0·9 c.c. gave a fall in blood-pressure only, which was roughly the equivalent of the fall produced by 0·3 c.c. of 1/40,000 histamine.

(ii) *Effect of beef extracts on the survival of adrenalectomized cats.*

The criteria used for testing the potency of the extracts prepared were: (1) effectiveness in prolonging the life of adrenalectomized cats beyond the ordinary limit of survival (11 days average, limit 31 days, Stewart and Rogoff [1929]; 7·7 days average, limit 16 days, Swingle and Pfiffner [1931 *a*]; (2) ability to restore to their normal strength cats suffering from severe prostration; (3) influence on symptoms of adrenal insufficiency, *e.g.* loss of appetite and weight, anorexia, vomiting, weakness and, in chronic cases, loss of hair.

Five cats adrenalectomized by the lumbar route, with an interval of at least 5 days between removal of the two glands, form the basis of our tests of the efficacy of extract of beef adrenal cortex. Of these one died at 11 days, a large wall abscess being found post-mortem. One died on the 45th day and another on the 57th day, both of them in spite of treatment. The remaining two died on the 62nd and 35th day, 6 and 5 days respectively after treatment was stopped. The lives of three other cats were prolonged by the use of the extract (Part II). Proof of the activity of the extracts by bringing about revival of cats prostrate from adrenal insufficiency is illustrated in cat No. 6.

Abbreviated Protocols.

Cat 1.

Second adrenal removed August 12. Weight 3·1 kg.

During the following 28 days received a daily average of 1·26 c.c. standard extract per day and lost weight steadily.

September 7. Weight 2·75 kg., appetite poor. More extract available, so received 3 c.c. a day subsequently and for 10 days appeared better and lost no weight. Caught cold September 17. In a week declined to 2·4 kg., hair fell out and appetite poor again. Purulent nasal discharge marked on September 24. Condition became progressively worse, weakness of limbs evident on October 6, cat prostrate on 7th, died next morning in spite of 20 c.c. extract in last 3 days. Weight 2·07 kg. Adrenalectomized life 57 days.

Autopsy. Foul, thick, yellow pus throughout nares and accessory sinuses. No accessory cortical tissue.

Cat 2.

Second gland removed August 14. Weight 2·3 kg.

During the following 19 days received a daily average of 1·5 c.c. extract, and gained weight.

September 2. Weight 2·37 kg. Condition good.

Dose same and weight same up to September 23; 6 days then of refiltered extract; lost 0·18 kg. weight over 3 days at end of that time but regained to 2·27 kg. on good extract. Extract stopped October 9. Normal for 4 days, then refused fish, vomited, appeared drowsy. Next day weak on hindlegs.

October 15, 8 a.m. Lying prostrate, convulsions, dead 8.45 a.m. Adrenalectomized life 62 days.

Autopsy. Negative. No accessory cortical tissue.

Cat 3.

Second gland removed September 1. Weight 2·25 kg.

Dressing came off wound 1 hour after operation. Appeared bright and well for 6 days; on 7th refused food, began to lose weight and died in spite of daily 2–2·5 c.c. extract. Dead 11th day.

Autopsy. Wall abscess.

Cat 4.

Second gland removed September 15. Weight 3·31 kg.

This animal was always sulky and easily frightened. Appetite always poor. Lost weight over first 28 days on 3 c.c. standard extract a day.

October 14. Weight 2·7 kg. Hair coming out. Dose raised to 5 c.c. a day. No improvement.

October 23. Weight 2·55 kg. Has eaten nothing in 4 days.

October 27. Sneezing and slight nasal discharge.

October 31. Dead. Weight 2·3 kg. Adrenalectomized life 45 days.

Autopsy. Stomach contains bile; several ulcers near pylorus. No accessory tissue.

Cat 5.

Second gland removed October 23. Weight 3·05 kg. Apathetic few days after operation. Swelling in region of wound; opened and lot of pus drained.

November 3. Weight 2·6 kg. Appetite better and cat more vigorous. Infection almost completely cleared up by November 5. Dose during this time 4–5 c.c. per day.

November 8. Weight 2·5 kg. Extract No. 24 (Mod. Ext.) administered for 10 days, 4 c.c. on 6th and 7th, 2 c.c. a day after.

November 16. Weight 2·41 kg. Daily dose 2 c.c. standard extract administered for a week.

November 22. Weight 2·47 kg. Extract stopped. Cat well and appetite good.

November 26. Vomited a little. No other obvious sign of insufficiency.

November 27. Found dead. Adrenalectomized life 35 days.

Autopsy. Stomach: extensive multiple ulceration at pyloric end and considerable extravasated blood.

Ileum: ulcer, 1 cm. in diameter half way down. No accessory cortical tissue.

See Fig. 1, Cat 6, in Part II for demonstration of revival from prostration.

It is apparent from the consideration of the protocol of cat 1 that 1·26 c.c. extract per day was insufficient to maintain good health. At this time difficulty was experienced in securing an adequate supply of glands, and by the time this was remedied the cat's condition had declined and the upper respiratory passages had become infected so that increased dosage was without effect. Although the amount of extract administered to cat 2 was little more, this animal did well. It was, however, a smaller animal. We are at a loss to explain the failure of cat 4 to do well, because no other animal has failed to respond to treatment with extract of beef adrenal provided severe wound infection had

not become established, as in cat 3. The failure of this animal to become accommodated to laboratory life may have been responsible, at least in part, for the unfavourable result. Cat 5 illustrates that infection can be overcome in adrenalectomized cats, by ordinary surgical measures, such as drainage, combined with increase in dosage of extract. The administration of smaller amounts of extract, 2 c.c. a day for comparative reasons, was compatible with life and health but was not accompanied by much gain in weight.

Further demonstration of the efficiency of extracts prepared in the laboratory is found in the restoration of strength in cat 6 by 8·5 c.c. of standard extract. This procedure was repeated on a subsequent occasion, 15 c.c. being used. This recovery was effected with one of the extracts mentioned as having been subject to extreme alkalinity.

Extract 24 prepared by the modified process of extraction does not appear to have been quite as active as ordinary stock extracts, since the weight of cat 5 declined while receiving this and rose subsequently on similar amounts of stock extract. The result is suggestive only that this method is no improvement on the original.

Whether the adjusting of the *p*H of the water to facilitate Seitz filtration improves the yield of active extract, we are not prepared to say, for there are not sufficient comparative data to decide the point. Our impression is that this procedure does improve the yield.

Discussion.

The very large amount of cortical tissue represented by the volume of extract necessary to maintain adrenalectomized animals in health has been remarked on by Britton and Silvette [1931] and others. Swingle and Pfiffner [1931 *a*] used 3 c.c. (corresponding to 150 g. moist gland) a day to maintain cats in good condition, though they say that this was by no means a minimal dose. More recent work indicates that 1/4 c.c. per kg. of body weight per day is the minimum maintenance dose for dogs weighing 10–15 kg. [Harrop, Pfiffner, Weinstein and Swingle, 1931]. In our experience 2 c.c. per cat per day is the lowest dose that has been used to maintain animals in good condition for any length of time. Since substantial evidence of the activity of the extracts only was desired and not accurate data as to their strength, further tests in this direction were not attempted. But it would appear that our extracts are not as potent as Swingle and Pfiffner's. This can only be attributed to the failure to have the glands cooled immediately on removal from the animals and is in accord with Britton and Silvette's experience.

Since adrenal cortical extract is being used clinically in Addison's disease and certain cases have failed to respond [Benham, Fisher and Thurgar, 1932; Levy Simpson, 1932], it is perhaps well to make reference to the published account of the comparative assay test of the commercial material. Of six cats treated with this extract [Wilson, 1931], two died of infections and one suddenly on the 16th day; these may be disregarded. The daily dose used in maintaining the remaining three cats for 30 days was 4 and 6 c.c.; presumably the gland equivalent was 50 g. to 1 c.c. The animals died in 3, 4, and 5 days after discontinuing injections of the extract, which is somewhat soon for well-treated cats. One can only conclude that the extracts contained only a fraction of the potency of Swingle and Pfiffner's original extract.

The demonstration of histamine in the extracts prepared by us leads us to believe that the severe reactions noted by Benham, Fisher and Thurgar were due to this. Commercial extract tested by us also contained histamine.

PART II.

Effect of extracts of elasmobranch interrenal body on adrenalectomized cats.

In view of the fact that endocrine gland products, such as adrenaline and insulin, obtained from as widely divergent sources as fish and mammals, show a remarkable specificity of action, it was thought that extracts of the elasmobranch interrenal body might exhibit the life-prolonging influence on the adrenalectomized cat that is shown by extracts of beef adrenal cortex.

Balfour in his monograph [1878] introduced the term "interrenal body" for the mass of lipoid tissue which is said to be homologous with the adrenal cortex of higher forms, *e.g.* Sharpey-Schafer [1924]. This "rod-shaped structure, paired in the rays" [Vincent, 1924] lies loosely attached to the posterior part of the kidney, separate from the segmentally arranged chromophile bodies. In consequence of this isolation the difficulty of getting rid of adrenaline in the preparation of extracts is not encountered as in beef gland extracts.

The fact that a large number of skate (*Raja clavata*) are daily brought to the Aberdeen fish market, made an attempt on this problem possible.

(i) *Material.*

The fish, gutted, except for the kidneys and adherent interrenal body, are removed from their ice packing on the trawler and landed

each morning at the fish market. There the kidney with the interrenal body was dissected out within 2 hours of landing. When as much as possible of this material had been collected it was brought to the laboratory and the interrenal bodies cut off with scissors and mixed immediately with the extraction fluid. Glands from fish of less than 2 ft. maximum breadth were so small as to be almost useless, but fish of 4–5 ft. wing tip measurement weighing upwards of a hundredweight, yielded about 6 g. of interrenal tissue.

(ii) *Methods of preparation of extracts of interrenal body.*

Method 1. *Batch* 1. The glands (52 g.) were cut finely with scissors, treated with acetone and placed in the refrigerator. The acetone was changed several times over a period of 4 days, 900 c.c. in all being used. The lemon-yellow acetone solution was filtered through a coarse paper, and distilled under reduced pressure at 45° C. to a volume of about 25 c.c. At this point 30 c.c. of distilled water of *p*H 5 were added, and the acetone further removed by distillation till a volume of 30 c.c. remained. When decanted from the distilling flask the watery solution had an orange supernatant layer. The flask was washed with 20 c.c. of water and the material now in 50 c.c. put through a Seitz filter.

Method 2. *Batches* 2–6. The Swingle and Pfiffner technique for the preparation of adrenal cortical extracts from beef glands has also been applied in the extraction of interrenal bodies.

Glands collected daily and weighed were ground in a mortar with 2·5–3 c.c. of 96 p.c. alcohol per g., and when a sufficient amount of material had been collected, over a period of 1–3 weeks, the alcoholic extraction fluid was removed from the combined lots of tissue. This was then pressed out, ground in a fine mincer and extracted for 2 or 3 days with 80 p.c. alcohol, 2 c.c. per g. This solution was then drawn off and the tissue pressed out. Each alcoholic solution was concentrated separately under reduced pressure at 45° to 1/15th of its original volume and the customary benzene and acetone extractions performed. Distribution between alcohol and petroleum ether was performed two or three times only, and the alcohol fractions washed with petroleum ether only once or twice. The material at each stage resembled outwardly that obtained from beef adrenals, with the exception of the acetone-soluble residue. This was syrupy as contrasted with the friable solid obtained from the beef glands at the same stage.

Method 3. *Batch* 7. Glands were collected over a period of 4 weeks. Each day's lot was ground in a mortar with 2·5 volumes of 96 p.c.

alcohol, and at the end of 10 days or 2 weeks the alcoholic extraction fluid was removed from the combined tissue which was pressed out and ground in a mincer. The tissue was then extracted repeatedly with ether, and the residue, after distillation of the alcohol under reduced pressure, similarly extracted with ether. The murky yellow ether solution was centrifuged to get rid of some suspended solids, and distilled under reduced pressure. The resulting brown syrupy residue was extracted for 7 hours with 500 c.c. of acetone in the refrigerator and similarly with a fresh 500 c.c. portion of acetone for another 15 hours. The residue, now less sticky, was transferred to a mortar and rubbed up with five 100 c.c. portions of ice-cold acetone. The acetone solutions were combined and distilled under reduced pressure at 45° C. The dark brown syrupy residue (about 20 c.c.) was taken up in 74 c.c. of 96 p.c. alcohol and 30 c.c. of petroleum ether and transferred to a separatory funnel. 26 c.c. of water were added. A second and a third distribution were performed with the petroleum ether and three washings of the alcoholic phases done with 30 c.c. portions of petroleum ether. The 70 p.c. alcohol solutions were then distilled under reduced pressure and by the aid of small portions of absolute alcohol reduced to a volume of about 25 c.c. The addition of 25 c.c. of water caused the brown solution to turn a milky orange colour, a lot of orange-coloured solid adhering to side of flask. This milky solution was decanted and made up to 40 c.c. All the latter operations were conducted in alcohol-washed vessels since, as a Seitz filter was not being used, it was desirable to have the extract as sterile as possible. Finally, the extract was put in a sterilized bottle and in this suspension form used for injection.

Summary of interrenal body extracts.

Method 1.	Batch 1.	52 g. 1 c.c. extract = 1 g. tissue.
Method 2.	Batch 2.	333 g. 1 c.c. extract = 5 g. tissue. Passed through alkaline permutit. No others put through permutit. Filtered through Seitz.
	Batch 3.	331 g. 1 c.c. extract = 6 g.
	Batch 4.	163 g. 1 c.c. extract = 4 g. No Seitz filtration.
	Batch 5.	635 g. 1 c.c. extract = 13 g. Seitz filtered.
	Batch 6.	222 g. 1 c.c. extract = 10 g. Seitz filtered.
Method 3.	Batch 7.	677 g. 1 c.c. extract = 16·5 g. No Seitz filtration.

(iii) *Effects of extracts of interrenal body.*

In endeavouring to decide whether extracts of elasmobranch interrenal body exert a beneficial influence on the survival of the adrenalectomized cat it has not been possible, owing to the limited supply of

interrenal tissue, to employ in full the criteria used with the beef cortical extract. Determination of its effect on the duration of survival of the animal has not been attempted, neither has the extract been administered to animals during the period immediately following adrenalectomy when the risk of death through causes other than adrenal insufficiency is high. During this doubtful period beef adrenal extract has been given and its administration continued until the condition of the animal showed that this risk of post-operative death was diminished.

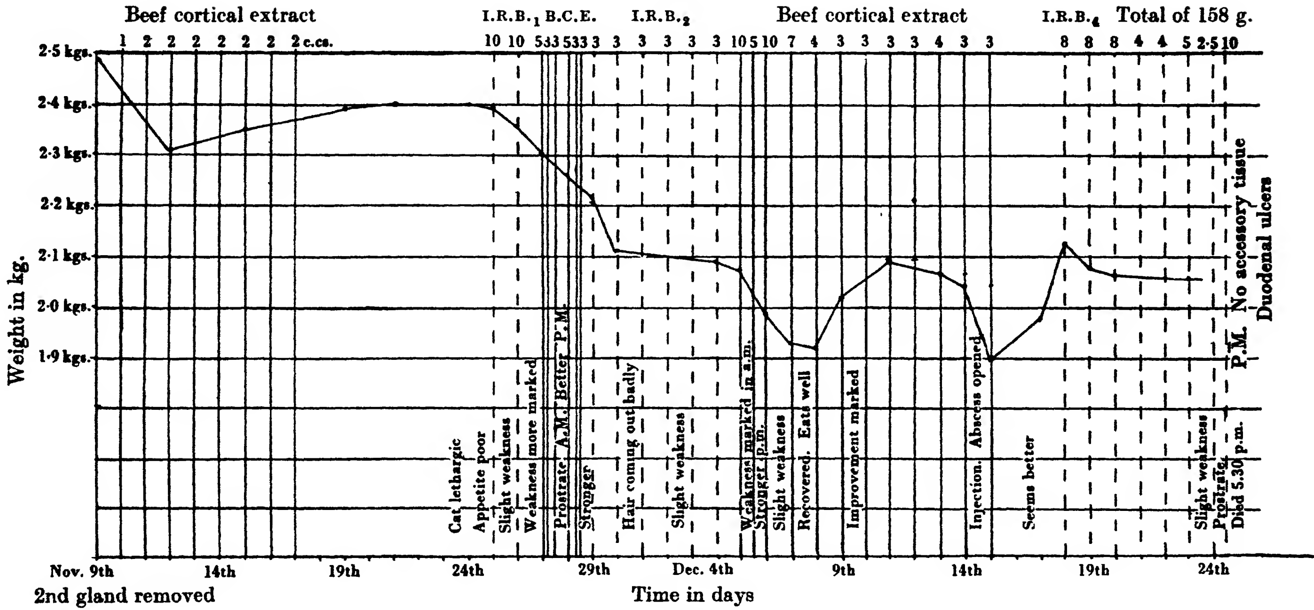
Beef extract has been used also, to restore animals suffering from severe adrenal insufficiency to a state of health permitting their use for testing other interrenal extracts.

Judgment of activity of interrenal extracts, then, has been based on the ability of this material: (i) to prevent the appearance of symptoms of adrenal insufficiency, (ii) to ameliorate the symptoms. Three adrenalectomized cats treated with beef extract as a preliminary have, after the withdrawal of this extract, been injected with interrenal extract. In each case symptoms of adrenal insufficiency have appeared within 2 weeks of cessation of the beef extract, as might be expected in the absence of interrenal extract administration.

The results of experiments are described below in the charts of Figs. 1–3. In these beef cortical extract is designated B.C.E.; interrenal body extract I.R.B., with number denoting preparation, *e.g.* from Batch 3 —I.R.B. 3.

These results show that in cat 6, extract of interrenal body, the equivalent of 20 g. of tissue, administered over 2 days, did not ameliorate the symptoms of severe adrenal insufficiency. When on the verge of death this animal was revived with beef cortical extract, but the subsequent administration of extract of interrenal body (the equivalent of 90 g. of tissue over 6 days) did not prevent the development of marked symptoms. On again administering beef cortical extract the cat regained its strength. Finally, administration of interrenal extract, the equivalent of 158 g. of tissue during 7 days did not prevent the development of severe symptoms, which terminated fatally in spite of increase in dosage.

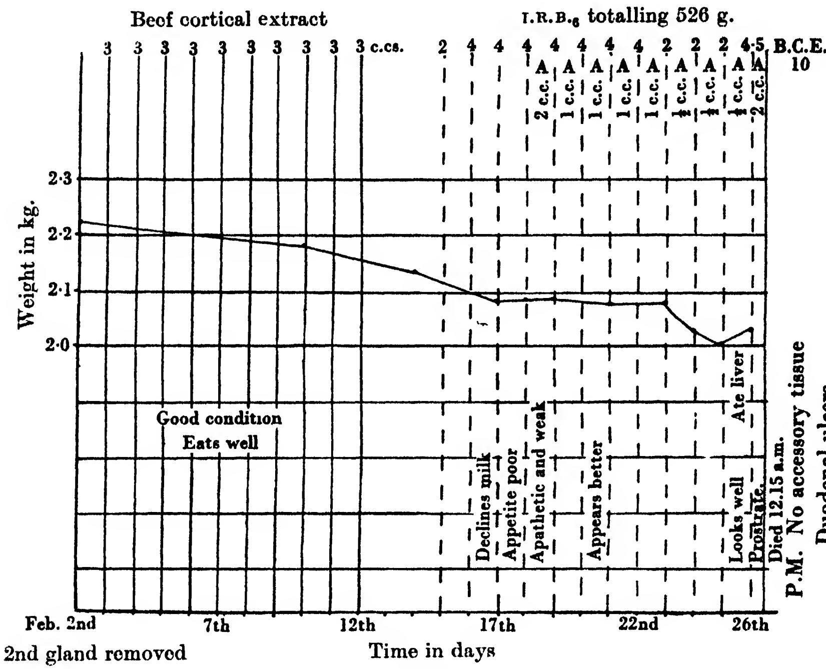
The same general result was obtained in cat 7. Here, however, adrenaline was added to the interrenal extract to make it more like beef cortical extract. Nevertheless, the cat died in 14 days after withdrawal of the beef cortical extract and the adrenaline did not seem to improve matters. The poor appetite and the weakness on the 17th were probably not due to adrenal insufficiency since the temperature remained normal. It will be observed that liver was fed to this animal, and it is possible,



Cat 6.

Fig. 1. Shows the body weight on the days indicated on the abscissa. The vertical continuous lines represent injections of beef cortical extract and the broken ones, of interrenal extract. The amounts of extract administered are represented by the figures at the top of the vertical lines. Abbreviations: B.C.E. Beef cortical extract; I.R.B. Interrenal body extract.

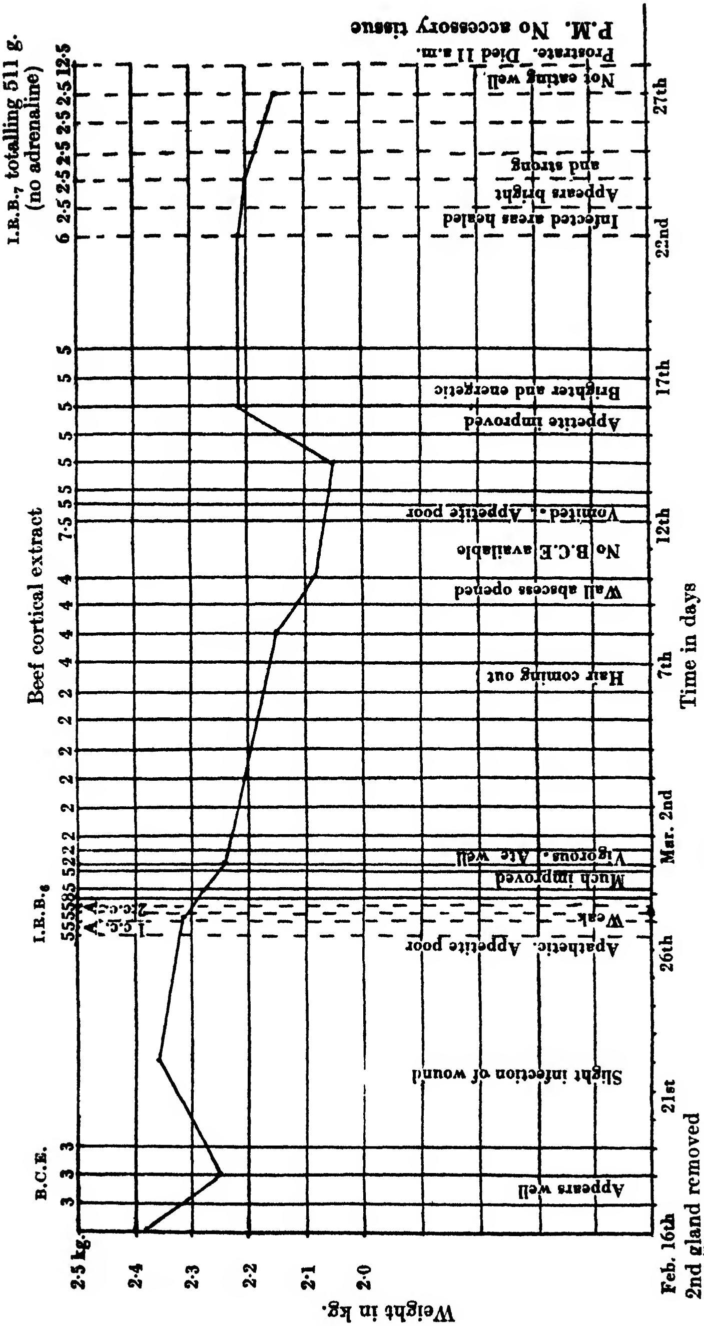
as has been noticed by Hartman, that this aggravates the symptoms of adrenal lack. The animal was comatose when beef extract was finally given, and did not recover. We have other reasons for believing that this particular beef extract was of inferior activity, and believe that our ordinary extracts would have restored the cat, even at this late stage.



Cat 7.

Fig. 2. Shows the body weight on the days indicated on the abscissa. The vertical continuous lines represent injections of beef cortical extract and the broken ones, of interrenal extract. The amounts of extract administered are represented by the figures at the top of the vertical lines. On the days marked "A" 1/500,000 adrenaline solution was injected in the doses indicated. Abbreviation as in Fig. 1.

The results obtained with these two animals are further confirmed by those with cat 8. The administration 7 days after withdrawing beef cortical extract of 20 c.c. I.R.B. 6, the equivalent of 200 g. of interrenal tissue, along with adrenaline over a period of 24 hours, did not improve the cat's condition which had become bad. When 8 c.c. of beef cortical extract were now given improvement was distinct within 12 hours, and it continued except for a setback due to abscess formation, during the 3 weeks that this extract was given, the body weight finally rising. Four



Cat 8.

Fig. 3. Shows the body weights on the days indicated on the abscissa. The vertical continuous lines represent injections of beef cortical extract and the broken ones, of interrenal extract. The amounts of extract administered are represented by the figures at the top of the vertical lines. On days marked "A" 1/500,000 adrenaline solution was injected in the doses indicated. Abbreviations as in Fig. 1.

days after discontinuing this extract, administration of I.R.B. extract was started, a total of 18·5 c.c., the equivalent of 305 g. of interrenal tissue being injected over a period of 5 days. This did not prevent the development of symptoms of adrenal insufficiency, noticeable by the loss of appetite, 9 days after the last injection of beef extract had been given. Severe insufficiency on the following day was not counteracted by increasing the dose of extract to 12·5 c.c.

Discussion.

It is apparent from these results that extracts of elasmobranch interrenal body contain no appreciable amount of the substance which, in extracts of beef adrenal cortex, prevents the appearance of the symptoms of adrenal insufficiency in the adrenalectomized cat or removes them if already established.

The explanation for this negative result may be either the absence from the interrenal body of the vital factor present in the homologous tissue of mammals, or deterioration of the active substance during the interval (several days) which elapsed between catching the fish and landing it. Although the fish were meanwhile kept on ice, it is possible that any active principle would be destroyed. If the interrenal bodies could be excised and placed in alcohol at sea, this source of possible error could be avoided. The only conclusion permissible from the present investigation is that the interrenal body, as removed from fish caught and transported to market in the usual way, is devoid of any of the principle present in beef adrenal which can greatly prolong the lives of adrenalectomized cats.

Summary.

1. Extracts of beef adrenal cortex prepared according to the method of Swingle and Pfiffner are shown to be effective in prolonging the life of adrenalectomized cats and in reviving cats suffering severe degrees of adrenal insufficiency. These extracts have been shown to contain histamine in very definite amounts.

2. Extracts of elasmobranch interrenal body prepared by various methods when injected into adrenalectomized cats have exhibited no apparent influence either in preventing the development of adrenal insufficiency within the anticipated time, or ameliorating the symptoms once these have appeared.

I wish to express my grateful appreciation of the helpful advice and criticism of Prof. J. J. R. Macleod, F.R.S., in the conduction of this work and acknowledge the assistance of my colleagues, particularly that of Mr J. M. Peterson, B.Sc. My thanks are also due to Dr J. J. Pfiffner for his kindly interest.

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DETERIORATION OF FIBRINOGEN AND THROMBIN.

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ACCURACY of determination of the time of onset of the coagulation of fibrinogen by thrombin *in vitro* depends upon avoidance of variation of technique and conditions of experiment, some of which, such as temperature, exact measurement of quantities of liquid used, *p*H of the coagulant mixture and degree of stirring, can usually be kept within limits too small to be of serious import, while others, such as deterioration of fibrinogen and thrombin on keeping, are not readily controlled. It is with the latter that the present investigation deals.

In the experiments about to be described citrated human plasma was used as source of fibrinogen (tri-sodium citrate being added in the proportion of 0·15 g. to 0·20 g. per 100 c.c. of blood withdrawn) and snake venom (*Echis carinatus*) as source of thrombin. The coagulation period, by which is meant the period of onset of coagulation, is the time after mixture of plasma and venom at which fibrin fibrils are first seen under dark ground illumination, this being capable of determination with much greater precision than is the appearance of "setting." For dilution of plasma and venom solution sterile 0·85 p.c. NaCl solution, with or without the addition of 0·10 p.c. of tri-sodium citrate, was used.

The stability of venom solution on keeping at 0° C. to 4° C. was first examined in the following manner. A 1 in 3000 dilution of venom in 0·85 p.c. NaCl solution was prepared. From this samples were taken at intervals, as required, and were further diluted to 1 in 320,000. In this dilution the coagulation period was determined with the aid of freshly prepared citrated human plasma, obtained from the same source in all experiments. The results are given in Table I: the figures in the third column represent the amounts of plasma contained in 1 c.c. of the mixture of plasma and venom solution employed for coagulation. These experiments, which were carried out at room temperature (17° C. to 20° C.), show that venom in 1 in 3000 dilution remained unchanged during the period of observation, namely 112 days for the first four experiments,

in which 48 p.c. of plasma were contained in the mixture of plasma and venom used for coagulation, and 600 days for the remaining experiments, in which 5 to 7 p.c. of plasma were present. The variations in the period of

TABLE I. Influence of age upon venom solution.

Exp. No.	Age of venom solution (days)	Concentration of plasma	Concentration of venom	Coagulation period (min.)
1	708	0·48	1 in 320,000	5·2
2	751	0·48	,,	2·7
3	782	0·48	,,	5·2
4	820	0·48	,,	3·5
5	222	0·05	1 in 2,560,000	18
6	378	0·07	,,	18
7	708	0·06	,,	29
8	751	0·06	,,	18
9	782	0·06	,,	27
10	822	0·06	,,	23

onset of coagulation given in the last column cannot be regarded as due solely to differences in the content of fibrinogen in the plasma, for slight changes in the conditions and technique of experiment are difficult to avoid in experiments extending over so long a period: the actual difference in titre of the samples of plasma employed is presumably slight.

TABLE II. Diminution of coagulability of plasma and venom solution on keeping.

Exp. No.	Age of venom solution (days)	Concentration of plasma	Concentration of venom	Coagulation period (min.)
1	0	0·48	1 in 320,000	3·5
2	9	0·48	,,	7·75
3	18	0·48	,,	9·5
4	31	0·48	,,	12·75
5	84	0·48	,,	16·25
6	0	0·06	1 in 2,560,000	23
7	9	0·06	,,	38
8	18	0·06	,,	99
9	31	0·06	,,	190

A further series of experiments was now made in order to determine the degree of change, if any, occurring on keeping. A sample of citrated plasma was prepared and, at the same time, a 1 in 160,000 dilution of venom, the coagulation period at room temperature (20° C. to 22° C.) of a mixture of the two in equal proportions being then determined. Similar determinations were also made with 1 in 8 dilutions of plasma and venom solution. The undiluted plasma and 1 in 160,000 venom solution were now stored in the cold room, samples being removed from time to time in order to observe any change in coagulation. The results, which are given in Table II and shown graphically in Fig. 1, afford evidence of

deterioration, a progressive increase in the period of onset of coagulation being observed, equivalent to a diminution of the content of fibrinogen or thrombin or of both. In order to form an estimate of the equivalent degree of diminution it is necessary to ascertain the effect on the coagulation period of lowering the amount of fibrinogen and thrombin, present in citrated plasma and solution of venom respectively, by dilution with

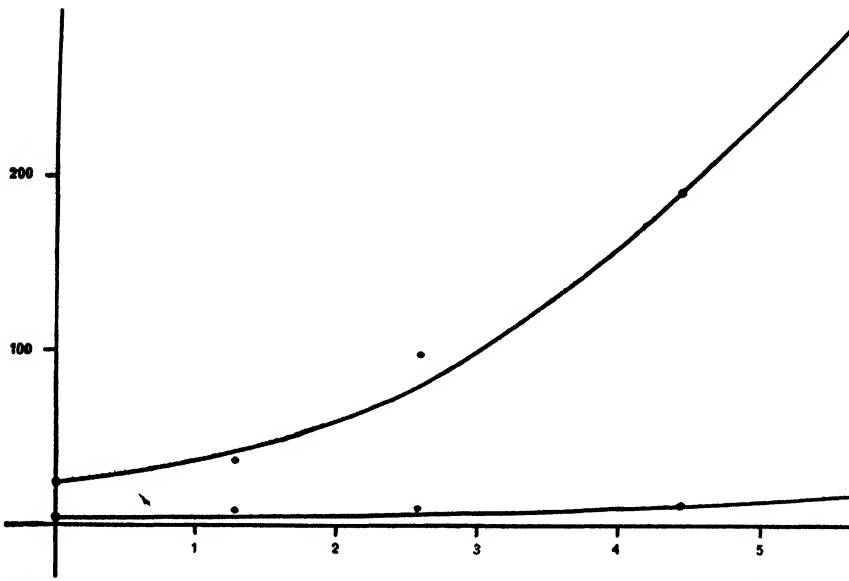

Fig. 1. Curves for experiments in Table II. Ordinates: period of onset of coagulation in minutes. Abscissæ: age of plasma and venom solution in weeks.

TABLE III. Effect of dilution of plasma and venom solution.

Exp. No.	Concentration of plasma	Concentration of venom	Coagulation period (min.)	Exp. No.	Concentration of plasma	Concentration of venom	Coagulation period (min.)
1	0·48	1 in 320,000	3·5	1	0·48	1 in 320,000	3·5
2	0·12	,,	5·75	5	0·48	1 in 1,280,000	10
3	0 03	,,	8	6	0·48	1 in 5,120,000	25
4	0·0075	,,	13	7	0·48	1 in 20,480,000	84
				8	0·48	1 in 81,920,000	200

0·85 p.c. NaCl solution. A series of experiments of this type, made immediately after preparation of plasma and venom solution, is given in Table III and the corresponding curves shown in Fig. 2 (in which relative, not actual, concentrations of plasma and venom are indicated on the scale of ordinates, the highest concentration being represented by 256 and successive dilutions by proportionately smaller numbers). By

means of these curves the degree of deterioration of fibrinogen and thrombin may be indicated. Thus the equivalent titre of fibrinogen in a sample of plasma is given on the curve to the left (Exps. 1–4) in Fig. 1 by the dilution of plasma corresponding to the coagulation period obtained when the sample is added to an equal volume of freshly prepared 1 in 160,000 venom solution. Similarly the titre of a solution of thrombin is represented on the curve to the right (Exps. 1, 5–8) by the dilution of venom corresponding to the coagulation period obtained when the

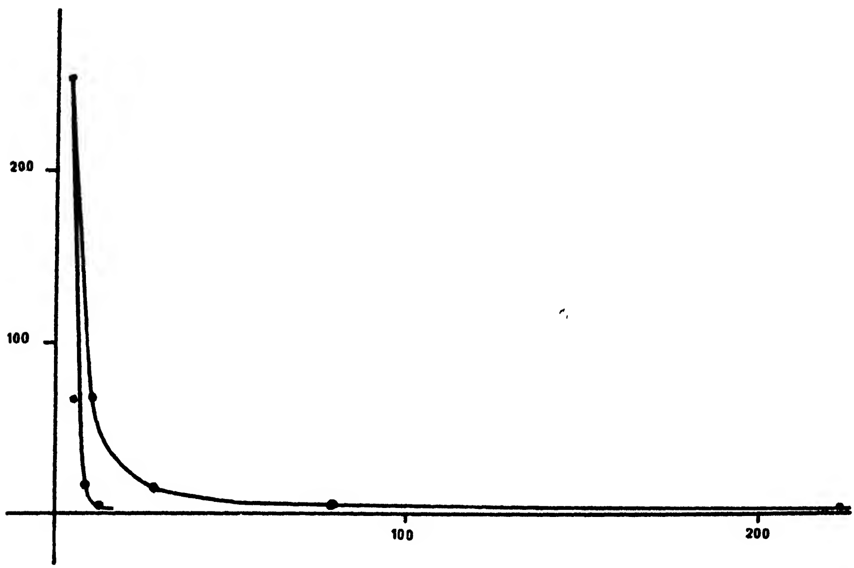

Fig. 2. Curves for experiments in Table III. Ordinates: relative concentration of plasma (curve to left) and venom (curve to right). Abscissæ: period of onset of coagulation in minutes.

solution of thrombin is added to an equal volume of freshly prepared citrated plasma, in each case fresh plasma and venom solution being taken as a standard. In this way the equivalent amounts of fibrinogen and thrombin at the end of 84 days in Exp. 5, Table II, were estimated to be in each case approximately 1/3·5 of the amounts originally present. Further determinations of titre, made at intervals with freshly prepared samples of plasma and venom solution giving initial coagulation periods (corresponding to Exp. 1, Table III) approximating closely to 3·5 min., are given in Table IV. An estimation of the range of concentrations of fibrinogen in Exps. 1–4 and 5–10, Table I, on the (incorrect) assumption that the values of the coagulation periods are due to variation in the

content of fibrinogen in the different samples of fresh plasma employed would give a ratio of highest to lowest concentration of 1 to about 1/4.

Both fibrinogen and thrombin deteriorate on keeping. Thrombin, however, is stable in relatively high concentration while fibrinogen cannot be kept unchanged even for short periods. Complete disappearance of fibrinogen was observed in old plasma, but old venom solution always retained some coagulative power. The rate of deterioration increases with rise of temperature and also with diminution of concentration: it is unaltered when the extent of the surface of glass with which fibrinogen and thrombin are in contact is considerably increased.

TABLE IV. Deterioration of fibrinogen and thrombin.

Material	Sample	Age	Titre reduced to
Plasma	A	9 days	1/3
,,	B	21 ,,	1/3
,,	B	40 ,,	1/9
,,	C	20 months	0
,,	D	20 ,,	0
Venom 1 in 160,000	b	21 days	1/5
,,	b	41 ,,	1/20
,,	a	41 ,,	No reduction
,,	e	4 months	,,
,,	f	14 ,,	,,
,,	g	25 ,,	,,

In all experiments the tubes and pipettes employed were sterilized, as were also liquids used for dilution. On examination under dark ground illumination no bacteria could be recognized in the coagulant mixtures, nor were fungi observed, though the latter occasionally appeared in very old plasma and venom solution. Attempted culture on serum agar was negative. The deterioration of fibrinogen and thrombin on keeping cannot therefore be attributed to contamination by bacteria or fungi.

SUMMARY.

1. Thrombin, contained in snake venom, remains unchanged in 1 in 3000 dilution. In higher dilution progressive deterioration occurs on keeping, but not complete disappearance.

2. Fibrinogen, contained in plasma, undergoes progressive deterioration on keeping, ultimately completely disappearing.

3. The deterioration cannot be attributed to the action of bacteria or fungi.

4. The degree of deterioration may be indicated by determining the amount of dilution of fresh plasma and venom solution respectively required to effect a corresponding increase of the coagulation period.

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THE INFLUENCE OF ELECTROLYTES ON THE FUNCTION OF THE INTESTINAL MUCOSA.

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We have shown that the selective action exerted by the epithelium of surviving intestine on sugar solutions placed in the lumen depends on the presence of Ca ions in the bathing fluid, so that when segments were immersed in Ca-free Tyrode the sugars passed through the intestinal wall in accordance with physical laws [Magee and Sen, 1931]. The present experiments were undertaken as an extension of this work, the objective being to determine whether the rate of absorption of glucose is related in any way to the level of Ca in the blood of the living animal. The results showed that this is so, and further experiments designed to elucidate the *modus operandi* of the Ca ions were then carried out. These took the form of observations on the villous movements, and the opportunity was taken to study their behaviour towards other ions of physiological importance.

The absorption experiments were conducted almost entirely on rats by the modified Cori method described elsewhere [Magee and Reid, 1931]. The rats used weighed from 150–250 g. Blood for Ca determinations was obtained when the rats were killed. If, as frequently occurred, enough blood for an estimation could not be obtained from a single animal, the blood of two or more was pooled. In all the absorption tests on rats the volume of the glucose solution (3–4 c.c.), its concentration (0·75 *M*), and the absorption period (30 minutes) were kept constant. As a preliminary to experimenting on animals with abnormal blood Ca, the coefficients of absorption (A.C.)[1] were determined in a series of normal animals, and the mean of these values was used as a standard for comparison with the other data obtained.

The mean A.C. for glucose in 15 normal rats was found to be 0·174 (Table I, line 1). This figure differs only slightly from that found by Magee and Reid, 0·167; a fact which, when considered along with the comparatively small standard error, 0·0048, affords good evidence for the

[1] To avoid confusion with the symbol for calcium we have decided to use the letters A.C. rather than C.A. to designate the coefficient.

TABLE I.

	Description of animals used	No. of animals	Solutions fed by stomach tube*	Blood Ca mg. per 100 c.c.	Mean absorption coefficient (A.C.)	Variations in A.C.	Standard error of mean A.C.	Increase + or decrease − p.c. over normal A.C., 0·174
1	Normal	15	0·75 *M* glucose	10·5	0·174	0·146–0·225	0·005	—
2	Normal	7	0·75 *M* glucose +0·32 p.c. $CaCl_2$	10·1	0·139	0·102–0·158	0·007	−22·9
3	Normal	11	0·75 *M* glucose +0·02 p.c. $CaCl_2$	10·6	0·208	0·154–0·243	0·009	+19·5
4	Parathyroidectomized	11	0·75 *M* glucose	7·1	0·106	0·060–0·133	0·006	−39·7
5	Parathyroidectomized	7	0·75 *M* glucose +0·32 p.c. $CaCl_2$	6·6	0·174	0·140–0·203	0·008	0
6	Deficiently fed, 30 days	1	0·75 *M* glucose	9·8	0·252	—	—	—
7	Deficiently fed, 30 days	1	0·75 *M* glucose	10·0	0·230	—	—	—
8	Deficiently fed, 51 days	5	0·75 *M* glucose	10·1	0·164	0·131–0·208	—	− 5·7

* 3 or 4 c.c. of each solution were fed at body temperature and assays were made of each solution.

TABLE III. Glucose absorption coefficient (A.C.) and blood sugar of luminalized rabbits before and during absorption of 40 c.c. 0 75 *M* glucose injected into duodenum.

Description of animals used	No. of animals	Mean blood Ca*	A.C.	Variations	Initial blood sugar mg. p.c.	Increase in blood sugar p.c. over initial at time in min. after injecting glucose						
						10	20	30	40	60	90	120
Normal	10	13·9	0·045	0·028–0·057	144	49	87	102	122	147	158	172
Parathyroidectomized	6	11·2	0·040	0·023–0·052	178	26	50	65	68	79	81	85
Deficiently fed	4	10·1	0·042	0	205	24	45	57	68	81	97	80

* The means were taken from 12 normal, 8 parathyroidectomized and 8 deficiently fed rabbits.

accuracy of the entire technique. The blood Ca was fairly constant and varied only from 9·6–10·6 mg. per 100 c.c. with a mean of 10·5 mg. It is well known that the blood Ca of mammals, although it maintains a remarkably constant level in health, is raised or lowered in some animals by excess or deficiency, respectively, of circulating parathyroid hormone. Similar effects are also produced by variations in the vitamin D and/or Ca content of the diet. We were not, however, aware of any experiments showing the applicability of these results to rats; so that, in the experiments about to be described, the influence of each factor on the blood Ca had to be determined.

Two methods of lowering the blood Ca were adopted, viz. parathyroidectomy and feeding on diets deficient in Ca and in vitamin D.

Absorption of glucose by parathyroidectomized rats.

The parathyroids in rats are embedded in the thyroid; so that, to remove the former, complete thyroparathyroidectomy had to be performed. The operation will be referred to for shortness as "parathyroidectomy" and the thyroparathyroidectomized animals as "operated." Blunt dissection was found to be more suitable for the complete removal of the gland and for controlling bleeding than cutting with sharp instruments. The wounds nearly always healed satisfactorily, sepsis being very uncommon. The most suitable time for carrying out absorption tests was found to be from 15–30 days after operation; because experience showed that the blood Ca was almost certain to be lowered by 2–4 mg. about two weeks after the operation; and because the number of deaths from tetany, although fairly frequent up to a month, increased rapidly after this time. Tetany was particularly liable to occur if an animal struggled unduly during the passage of the stomach tube.

The results (Table I, line 4) give the mean values for the number of animals stated in column 3. They show that the A.C. in the operated rats was 0·106 compared with 0·174 for the normal animals—a decrease of 39·7 p.c. The blood Ca was, on the average, about 3 mg. below the normal level. This was undoubtedly due to the parathyroidectomy; but the decreased A.C. could not be taken as related causally to the subnormal blood Ca, until the influence of two possible complicating factors was eliminated. The first was, that a condition of hypothyroidism as well as one of hypoparathyroidism existed in all the operated rats. Some of these animals were given 0·05–0·1 g. dried thyroid per rat daily from the day after the operation until the absorption tests were carried out. As the A.C. showed no sign of being increased thereby it was concluded that

hypothyroidism could be ruled out as a complicating factor. The other factor to be considered was a slowing of the circulation rate which might conceivably result from the parathyroidectomy. In order to control this possible source of error, observations were made on the effect of adding a calcium salt to the glucose solution administered to operated animals. The real objective of these experiments, however, was to ascertain whether the slow rate of absorption in rats with subnormal blood Ca could be improved by the presence of Ca ions in the intestinal lumen.

A concentration of 0·32 p.c. $CaCl_2$, which was believed to be roughly equivalent to the diffusible Ca in rats' milk, was incorporated in the glucose solution. This figure was arrived at by calculation from the data of Magee and Harvey [1926] for cow's milk, allowance being made for the fact that the salt content of the milk of mammals increases in amount with the rate of growth of the species. It was obviously essential for purposes of comparison to ascertain the effect of this concentration of $CaCl_2$ on the absorption of glucose in normal rats. A solution containing 0·32 p.c. $CaCl_2$ and 0·75 *M* glucose was, therefore, administered to normal and operated rats and the A.C. of glucose determined.

The average results are given in Table I, lines 2 and 5. In the normal rats the mean A.C. was 0·139, *i.e.* a depression of almost 23 p.c. as compared with the value for the controls, 0·174. In the operated rats, on the other hand, the mean A.C. was 0·174, which is identical with the value found in the controls. When, however, this A.C. is compared with the mean for the operated animals which were fed on glucose only, 0·106, it is seen that the rate of absorption was raised by 39·7 p.c. as a result of the addition of 0·32 p.c. $CaCl_2$.

These results suggest that the Ca ion exerts an apparently specific effect on glucose absorption, which can be brought about either from the intestinal capillaries or from the lumen. The blood Ca in all the operated rats was distinctly subnormal; and it has been seen that, when Ca ions were not administered along with the glucose to these animals absorption of the sugar was depressed, whereas when Ca ions were present in the glucose solution, absorption proceeded at the normal rate. It would therefore seem that the Ca ions, operating in the lumen, were able to counteract the defective absorbing powers of the intestinal epithelium which resulted from the deficiency of Ca ions in the circulating blood.

The above findings also suggest that, if glucose absorption is to proceed at an optimal rate, there must be an optimal concentration of Ca ions in the blood and/or in the intestinal lumen. The question as to the optimum for the blood is dealt with below; and, as regards the lumen,

it is clear from the inhibitory effect of 0·32 p.c. $CaCl_2$ in normal rats that the optimum is at a lower concentration than this. As a step towards the settlement of this matter the effect of incorporating 0·02 p.c. $CaCl_2$ in the glucose solution on the absorption of the sugar was tested. This concentration of $CaCl_2$ is identical with that present in Tyrode's solution.

The results are given in Table I, line 3, and they show that the average A.C. was 0·208, *i.e.* an increase of 19·5 p.c. over that of the controls (0·174). It is clear from these data that the presence of Ca ions in the intestinal lumen is necessary for optimal absorption of glucose to proceed.

The foregoing results raise the interesting question as to the cause of disordered nutrition observed in animals fed on diets deficient in Ca or in the factors which control its absorption. It may be that the condition is due ultimately to failure to absorb adequate amounts of constructive and energy-giving materials rather than to disorganized metabolism of them after absorption. If this conception is true the low blood Ca in such animals may probably be regarded as the primary manifestation of the defective nutrition.

Glucose absorption in rats previously fed on a diet poor in Ca and in vitamin D.

A group of rats was placed on a diet of equal parts of barley and wheat grains plus distilled water. After 30 days the A.C. and blood Ca of two rats were determined. The blood Ca was not appreciably affected and the A.C.'s were slightly above the range for normal rats (Table I, lines 6–8). Three weeks later five more rats were tested. Again, the blood Ca showed no clear evidence of a fall and, although the A.C. was lower by about 6 p.c. than the normal value (0·164 as compared with 0·174), the difference cannot be regarded as significant. The results also gave the impression that it is not possible in rats to induce a marked fall in blood Ca by a diet of this type, without reducing the animals to an extreme state of malnutrition, which would itself introduce other complications. The prosecution of this line of enquiry was, therefore, dropped.

Effects of hypervitaminosis-D and of parathormone on glucose absorption.

The objective of these experiments was to determine whether the blood Ca of rats could be raised by overdosage with vitamin D or by hyperparathyroidism and, if this transpired, whether the rate of glucose absorption was thereby accelerated. In addition, it was hoped that the

enquiry would give some information on the matter of the optimal blood Ca level for absorption of glucose.

A group of rats was fed on a diet complete in all known constituents plus two drops of radiostol per day administered to each rat by means of a pipette. After 18 days, absorption tests were carried out, using 0·75 *M* glucose only. The result (Table II) was an A.C. within the normal range

TABLE II. Rats fed on vitamin D in excess.

Days	Blood Ca	A.C.	Parathormone
18	11·1	0·155	0
20	11·0	0·200	0·2 c.c.
20	9·8	0·236	,,
20	9·7	0·220	,,
21	12·0	0·186	,,
21	12·6	0·228	,,
21	11·8	0·182	,,
21	12·4	0·164	0·25 c.c.
25	10·6	0·170	0
25	11·0	0·228	0
25	10·6	0·204	0
25	11·4	0·184	0
25	11·0	0·155	0
25	10·6	0·173	0
26	9·6	0·210	0
26	9·7	0·216	0
Average	10·9	0·194	
Standard error	—	0·007	

Increase over normal A.C. (0·174) 11·5 p.c.

and a blood Ca value very slightly above the normal mean. Fifteen hours before the next six tests the rats received subcutaneous injections of 0·2–0·25 c.c. parathormone, the potency of which had previously been demonstrated on a dog. No consistent increase in blood Ca occurred; but the A.C.'s were, on the whole, slightly raised. As parathormone appeared to be without effect no further injections were given. The findings in the remaining animals showed, on the whole, a slight increase in the A.C. as compared with the mean for the controls (0·174) but no consistent raising of the blood Ca. The means of all these results gave a value for blood Ca (10·9 mg.) slightly, but not significantly higher than the mean for the controls (10·5) and an A.C. of 0·194, which is 11·5 p.c. higher than the control average (0·174). This difference was found to be "possibly just significant" statistically, and so is suggestive of a slight increase in the rate of absorption. Before any definite conclusion could be given, however, the experiments would have to be repeated on animals whose blood Ca is known to be raised by parathormone or by vitamin D in excess. Until such data are obtained the question as to whether the normal blood Ca level is the optimum for glucose absorption cannot be answered. Although

the blood Ca was not consistently increased in the above experiments, this fact does not necessarily exclude the possibility of increased absorption of Ca, which could be offset by increased deposition in the tissues or by increased excretion. The former alternative would seem the more probable; for there is abundant evidence to show that vitamin D or ultra-violet radiations increase the absorption and retention of Ca. Since it has been shown that glucose absorption is promoted by feeding simultaneously 0·02 p.c. $CaCl_2$, it is conceivable that the acceleration in the rate of glucose absorption shown in the present series of experiments was due to stimulation of the absorptive activity of the mucosa by the passage through it of amounts of Ca above the normal.

Experiments on rabbits.

Experiments were carried out on rabbits in order to obtain a more accurate picture of the relationship between the blood Ca level and the rate of glucose absorption. These animals were selected because on them repeated determinations of blood Ca can be readily and accurately carried out. The procedure adopted was to determine the course of the systemic blood sugar (carotid or jugular) in anæsthetized animals during absorption of 0·75 *M* glucose injected into the duodenum, the A.C. being determined when the animals were killed at the end of the experiment. The method was almost identical with that used in the experiments of Magee and Reid. Rabbits with normal and lowered blood Ca were used, parathyroidectomy or a diet of wheat and barley plus distilled water being employed to lower the blood Ca. The two parathyroids which lie at a variable distance from the thyroid and the two lobes of the latter which include the remaining parathyroid tissue, were removed; the isthmus being left intact in order to avoid athyroidism. Almost invariably the blood Ca was found to have fallen by 2–4 mg. about 30 days after the operation. A month to six weeks on the deficient diet generally sufficed to cause the blood Ca to fall by 3–4 mg. Only when such a fall had been established were absorption experiments performed. These formed two series: the first, in which we had the cooperation of Mr F. J. Elliot, B.Sc., were done with luminal anæsthesia: the second set with amytal anæsthesia.

The results (Table III, p. 434) were not conclusive, and so will only be referred to briefly. The mean A.C. was higher in the normal than in either of the other groups, but this finding cannot be taken as more than slightly suggestive in view of the wide variations. No more exact valuation can be placed on the blood sugar data. These, although they show that the

percentage rise was greatest at every interval in the normal animals, were vitiated by the fact that the initial blood sugar in the two groups with subnormal blood Ca was already considerably raised. The latter animals responded so badly to luminal, and especially to amytal, anæsthesia that more than half of the experiments begun on them broke down because of the poor condition of the animals. These drugs depressed the blood-pressure markedly. Even in the successful experiments the blood-pressure was rarely much above 50 mm., a level which was considered as the minimum essential for the dependability of the experimental findings. Although these experiments failed to give any reliable information on the problem we set out to solve, they are, we think, worthy of record, in that they demonstrate a marked susceptibility to the toxic effect of barbiturates in animals with subnormal blood Ca; and, furthermore, because they show that the blood Ca of rabbits can be lowered by parathyroidectomy or by Ca-poor diets.

Action of electrolytes on villous movements[1].

These experiments, in which Mr E. A. Horne, B.Sc., cooperated, were carried out according to the technique outlined by Magee and Reid. Cats were employed, some of them being decerebrated in order to test the opinion of King and Arnold [1922], that better movements are obtained with decerebrate than with anæsthetized animals. Contrary to the observations of these workers we found that movements were just as good in luminalized as in decerebrate cats. Further, the slow respiratory movements of animals desensitized with this drug were found to be more conducive to accurate observations than the short rapid movements of animals under urethane or ether anæsthesia.

The effects of electrolytes on the villous movements were studied according to three schemes of examination. (1) Modifications of Tyrode's solution were applied to the mucosa and the effects compared with those elicited by previous and subsequent applications of normal Tyrode. (2) In the same way the effects of isotonic solutions of salts were compared with those of normal Tyrode. (3) Isotonic solutions of salts having a common anion or cation were applied successively and the influence of separate anions or cations compared. As all the solutions employed were practically iso-osmotic, complications due to differences in osmotic pressure could not arise. No observation was recorded until after at least

[1] The binocular microscope used for these experiments was kindly lent to us by Prof. Ritchie, Natural History Department, University of Aberdeen, to whom our best thanks are due.

three applications of any solution to the mucosa. As the movements of the muscularis mucosæ showed little constancy in their response to the various solutions only the effects on the pumping movements of the villi are recorded. All parts of the small intestine were studied, except the upper portion of the duodenum which, in the cat, is fixed to the posterior abdominal wall.

(1) *Modifications of Tyrode: comparisons made with Tyrode.*

*p*H 2·4–3·0. Stimulation at all levels, often temporary; shrinkage of villi; secretion of mucus.

*p*H 3·6–4·6. Indifferent in duodenum; inhibitory in ileum.

*p*H 5·6–7·4. Indifferent at all levels.

*p*H 9·2–9·6. Inhibition at all levels, but less marked in ileum.

KCl-free. Indifferent in duodenum; tendency to inhibition in ileum.

0·05, 0·1 and 0·2 p.c. KCl. Indifferent at higher levels; tendency to inhibition at lower levels.

$CaCl_2$-free. Inhibition at all levels.

0·05 and 0·1 p.c. $CaCl_2$. Indifferent at all levels.

0·2 p.c. $CaCl_2$. Inhibition at all levels.

N.B. Normal Tyrode contains 0·02 p.c. KCl and 0·02 p.c. $CaCl_2$ and the *p*H is 7·4–7·6, which was the *p*H of all the above solutions unless otherwise stated. "Indifferent" means that the effect did not differ from that of normal Tyrode.

(2) *Isotonic solutions: comparisons made with Tyrode.*

NaCl. Indifferent at all levels.

KCl. Indifferent at all levels; slight tendency to stimulation in duodenum.

$MgCl_2$. Very marked inhibition at all levels and very slow recovery.

$Na_2HPO_4 + NaH_2PO_4$. Marked stimulation at all levels with very big amplitude.

Na_2SO_4. Marked stimulation at all, especially at higher, levels.

$FeSO_4$. Marked stimulation at all levels; shrinkage; secretion of mucus; discoloration due to precipitation of Fe.

N.B. The *p*H of the above solutions was 6·9–7·1 except $FeSO_4$ which was 6·5. The effects, according to the results under (1), were not, therefore, due to variations in reaction.

(3) *Isotonic solutions: comparisons of salts with common anion or cation.*

Decrease of motility in order shown (= indicates same effect).

KCl, NaCl and $CaCl_2$. Duodenum—K > Na > Ca. Jejunum and ileum—K = Na = Ca.

Na_2SO_4 and $FeSO_4$. All levels—Na = Fe, but Fe caused marked shrinkage and secretion of mucus.

NaCl, Na_2SO_4 and $Na_2HPO_4 + NaH_2PO_4$. All levels—$PO_4 = SO_4 > Cl$; movements due to PO_4 more extensive but slower than those due to SO_4.

N.B. The *p*H of the solutions was as in (2).

The stimulation of pumping elicited by Tyrode of *p*H 2·4–3·0 is noteworthy, as also the secretion of mucus brought about by this solution

and by $FeSO_4$. The latter was most probably a protective phenomenon as Magee and Southgate [1929] have suggested. All parts of the intestine were insensitive to changes in *p*H between 3·6 and 7·4 except the ileum where the indifferent range was from 4·6 to 7·4. Markedly alkaline reactions (*p*H 9·2–9·6) caused inhibition generally; but it was less marked in the ileum, which, it is interesting to note, is, in life, subjected to more alkaline reactions than the duodenum. The duodenal region, on the other hand, most probably encounters reactions as acid as any used in these experiments [*vide* McClendon and Medes, 1925]. It will also be seen that, whereas the range *p*H 3·6–4·6 had no characteristic effect on the higher levels it caused inhibition in the ileum.

The observations under the above three headings for NaCl, KCl and $CaCl_2$ appear to be contradictory. Thus, from schemes (1) and (2), it would appear that KCl was not necessary for movements, and from (3) that it was more favourable to movements in the duodenum than $CaCl_2$, which appeared from (1) and (2) to be somewhat more essential for movements than NaCl or KCl. These varying responses were doubtless due to the different procedures employed. Thus, in scheme (1), the effects produced could not be attributed entirely to the salt whose concentration was altered. They were rather the effect of this change as modified by the simultaneous presence of other physiologically active ions. In perhaps a similar fashion under scheme (2) the characteristic action of these salts was affected by the previous treatment of the mucosa with Tyrode. It is also noteworthy that the observations under (1), and to a lesser degree under (2), were by far the most difficult to make, because the effects were very seldom of a striking nature.

There is no clear relationship between the effects of Ca on the villi and on absorption. Had the Ca ion, like the PO_4 ion, excited vigorous pumping movements a definite correlation would have been established; but, since such an effect was entirely absent, one must conclude that the observations on the villous movements throw no light on the manner in which Ca ions control absorption of glucose.

The effects of $MgCl_2$ were so very striking and so constant that it was not considered necessary to study this salt by any other scheme than (2). Pumping was almost at once brought to a complete standstill, and recovery afterwards with Tyrode was very slow.

Equally marked and consistent were the effects of phosphate and sulphate. These anions excited by far the most vigorous and lasting pumping movements of any solutions we have examined. The most striking movements were elicited by the PO_4 ion. These were of very

great amplitude and, consequently, were very conspicuous. In the similar experiments of Magee and Reid the phosphate solutions employed were very weak in comparison, and there is no doubt that the swelling and mucous secretion which were noted were due to the hypotonicity. It is not improbable that vigorous movements were excited even by these weak solutions, although it was impossible to see them owing to the covering of mucus. A clearing up of this doubt would provide some insight into the mechanism by which phosphates stimulate absorption of glucose, as shown in the experiments of Magee and Reid.

It was also found by these workers that 0·75 *M* glucose had a better effect on villous motility than any other solution of glucose or of any other sugar: a result which suggested an effect specific for glucose. To test this point 0·75 *M* glucose and iso-osmotic NaCl (2·65 p.c.) were compared in this regard. No constant difference between their effects was discernible, and it would, therefore, appear that the effect of 0·75 *M* glucose was osmotic and not specific in nature.

Summary.

The absorption coefficient for 0·75 *M* glucose was determined in rats under various conditions. The coefficient for pure glucose was 40 p.c. lower in parathyroidectomized rats with subnormal blood Ca than in normal rats. This depression was completely absent in similar animals when the glucose fed contained 0·32 p.c. $CaCl_2$. A concentration of 0·32 p.c. $CaCl_2$ in the intestine of normal rats depressed by 23 p.c., while one of 0·02 p.c. $CaCl_2$ increased by 20 p.c., the absorption coefficient of glucose. A certain concentration of Ca ions in the environment of the intestinal epithelium is therefore considered necessary for optimal absorption of glucose. Prolonged over-feeding of vitamin D and single parathormone injections did not significantly increase the blood Ca, and absorption of glucose in animals thus treated was only slightly increased. Neither the blood Ca nor glucose absorption was appreciably influenced by prolonged administration of diets deficient in vitamin D and Ca.

The influence of electrolytes on the movements of the intestinal villi in the cat was investigated. Tyrode at *p*H 2·4–3 excited vigorous movements in all parts of the intestine, while at *p*H 9·2–9·6 it depressed them. Tyrode of intermediate reactions had almost the same effect as normal Tyrode. The stimulating influence of ions on villous movements decreased in order of the following series: $K > Na > Ca > Mg$ in duodenum, $K = Na = Ca > Mg$ in jejunum and ileum; $Na = Fe$ at all levels;

$PO_4 = SO_4 > Cl$ at all levels. The effects of K, Na and Ca were modified by the presence of the other ions in Tyrode. No relationship could be found to exist between the effects of Ca ions on glucose absorption and on villous motility.

The blood-pressure of rabbits with subnormal blood Ca was markedly decreased by luminal or amytal anæsthesia.

We desire to express our thanks to Dr J. F. Tocher for instruction in statistical methods, to Prof. J. J. R. Macleod for criticism and to the Eli Lilly Co., Indianapolis, U.S.A., for the supply of parathormone and amytal used in the research.

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IDENTIFICATION OF LUCAS'S α EXCITABILITY.

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FROM the experiments of Lucas [1907–8], Jinnaka and Azuma [1922–3], Davis [1922–3] and Watts [1924–5], it appeared that the chronaxie of muscle might assume values very different from that of the nerve. These results were formerly contested by Lapicque and his followers, but as the results of recent experiments made by Lapicque and myself, there is complete agreement on the following points:

(*a*) In certain circumstances a strength-duration curve can be obtained whose time relations are much longer than that of the nerve. This curve is called α (after Lucas).

(*b*) The conditions for obtaining α may be secured by using large fluid electrodes, especially when the cathode falls at the extremity of the muscle.

(*c*) Conditions giving rise to an α curve do not necessarily exclude an excitability (γ) with excitation time (chronaxie) the same as that of nerve. Thus the strength-duration curve may be made up of these two curves meeting at a kink (see figures in former papers, Rushton [1930, 1931]).

With regard to the γ curve which obtains in these circumstances I have recently [1932 *c*] attempted to identify the structure responsible for it with some histological element in the muscle. A very brief summary of this and other investigations relating to the present problem is given later in this paper, suffice it here to state that various kinds of excitability relations, the effect of curare, and of excision of some of the nerve twigs, all unite to indicate that the γ curves arise from the direct excitation of the intramuscular nerves.

This analysis was conducted throughout with large fluid electrodes, and the conclusions are not necessarily valid when applied to results

with needle or capillary electrodes. But in those circumstances where it is possible to obtain an α curve, it appears that the γ curve is there always due to nerves, and the question next arises as to the identity of α. The conclusion that α is due to the excitability of the muscle fibres themselves is so obvious that it requires no very detailed discussion, but we are still faced with the question as to whether the excitation in this case may not be qualitatively of a different kind from that in nerve and in muscle when small electrodes are used.

I am indebted to Prof. Lapicque and to Prof. Adrian for pointing out to me two possibilities which bear upon this matter:

(i) Is the α excitability due to the excitation at the opening of a constant current?

(ii) Is the α excitability associated with the local cathodic contracture and not with the propagated disturbance?

It will therefore be convenient to consider these various questions under separate headings as follows:

Part I. Identification of the α excitability with the muscle fibres.

Parts II and III. Examination of the two above-mentioned possibilities.

This paper concludes the series in which I have attempted to identify the α and γ substances without the use of drugs, fatigue or other abnormal physiological conditions which might alter the chronaxie. In order to bring together the results of this study, Part IV of this paper will include a summary of the chief experimental results together with such conclusions as seem legitimate. Finally in Part V a hypothesis will be advanced to account qualitatively for the great effect of electrode size upon the excitation time of muscle.

Part I. Identification of the α excitability.

Since the γ excitability has been identified with nerve twigs, the presumption that the α excitability is muscle is so great that a detailed investigation as in the case of the γ fibres would be tedious. With hardly any new experiments we may summarize certain conclusions from former papers.

(*a*) The α excitability is found in the nerve-free pelvic end of the sartorius, it persists after curare, and has an excitation time (chronaxie) 10–100 times that of nerve, and therefore it is not nerve or nerve ending.

(*b*) The threshold-angle results [1930, 1932 *c*] show that the α fibres run in the direction of the muscle fibres.

(*c*) The muscle fibres contracting to an α stimulus account for practically all the tension developed by a twitch.

(*d*) These muscle fibres are in a normal condition in that they are supplied by nerves, are found in all muscles, and in all states of survival of the muscle from the intact condition in the spinal animal to the equilibrium state after remaining 24 hours in Ringer's fluid.

(*e*) Bremer has shown that in certain circumstances a muscle (frog's gastrocnemius) may exhibit a contraction very different from the familiar twitch. This phenomenon, which he calls "neuro-muscular contracture," is characterized by the fact that the contraction is very slow, the tension developed slight and the excitation time of the underlying excitability long. He finds in fact that the excitation time is about a hundred times as long as that for the motor nerve [Bremer, 1930, p. 315], which is just the order of magnitude of the α excitation time. The question therefore arises as to whether the α contraction is to be identified with the neuro-muscular contracture.

It has already been shown [Rushton, 1932 *a*] that the tension time of the α contraction is the same as that of the ordinary twitch, whence it was concluded that the α contraction was an ordinary one. If this is correct α cannot be a neuro-muscular contracture which is much slower and weaker [Bremer, 1930, p. 315]. But since it is conceivable that Bremer's contracture might have the same tension time as a twitch—gaining in duration what it lost in intensity—tension time alone will not definitely exclude the identification with the α contraction; for this exclusion an isometric α record is required. In a former paper [Rushton, 1931] it was remarked in a footnote that the isometric twitch was identical whether the excitation was through the nerve or the α substance. The experimental evidence in support of this statement has not been presented and is as follows.

The sartorius muscle was set up as usual in a trough with fluid electrodes of the "block" type applied to the pelvic end with the anode towards the pelvis. The tibial tendon was attached to a rather stiff insensitive tension lever writing on a smoked drum. A strength-duration curve was obtained as shown in Fig. 1 plotted in double logarithmic coordinates. The curve is obviously composed of γ and α portions which may be extrapolated as shown with fair accuracy. Five isometric records were now taken, when the direct stimulus to the muscle was of strength and duration indicated by the triangle, circles and crosses in Fig. 1. It is seen that the crosses are above the γ threshold but below the α, whereas the remainder are above the α threshold but below the γ. Fig. 2

shows the five isometric twitches arising from these stimuli. The amplitude of the tracings was small and Fig. 2 gives the result of measuring the records under a lens, scaling to a common maximum and replotting.

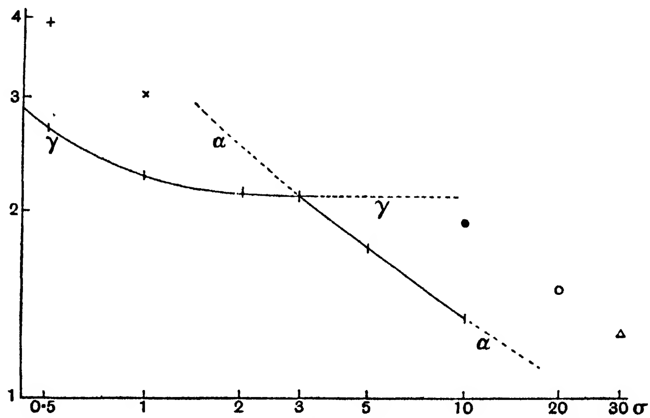

Fig. 1. Strength-duration curve of sartorius. Ordinates, current strength in arbitrary units scaled in logarithms; abscissæ, durations in σ scaled in logarithms.

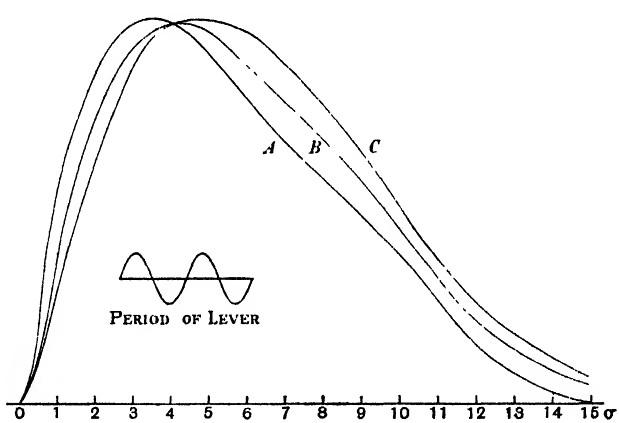

Fig. 2. Isometric twitches of muscle of Fig. 1. *A*, *B*, *C*, when stimuli were crosses, circles and triangles respectively (Fig. 1). Abscissæ: time in σ. Inset: natural period of lever with muscle attached.

The curves from the two stimuli indicated by a cross in Fig. 1 were practically identical (*A*), also the two circles gave nearly coincident curves (*B*), the triangle is represented by *C*. The natural period of the lever with muscle attached is inset. It is seen at a glance that to a first approximation the isometric twitch is identical whether elicited through

γ or α, and it is quite out of the question to suppose that the α contraction has the time course of Bremer's contracture. With regard to minor details in Fig. 2 it is seen that the longer the duration of the stimulus the later lies the maximum and the relaxation phase of the curve. This is easily accounted for by the fact first that the branching nerve fibres cause the γ stimulus to spread quickly all over the muscle, whereas the α excitation is propagated relatively slowly along the muscle fibres. Second, that when the duration of the stimulus is long the various fibres are not all excited at the same moment. Consider, for instance, the strength and duration represented by the triangle (Fig. 1). When this current has only lasted 12σ the threshold fibres respond, and more and more fibres enter over the next 18σ. Clearly this scatter in the moments of reaching the threshold will cause just the observed kind of modification in the shape of the isometric twitch.

Thus in spite of the crudeness of the lever employed it may safely be concluded that the twitch has the same time course whether elicited from an α stimulus or a γ one (*i.e.* through the nerve), and since the tension time is also the same, so must be the tension developed.

The α contraction therefore cannot be the same as Bremer's neuromuscular contracture, since both the time course and the tension developed are of quite different orders of magnitude.

We may thus conclude that the α excitability is that of the ordinary muscle fibres, that all the muscle fibres exhibit this excitability, and that the fibres are in good physiological condition.

PART II. IS THE α CURVE DUE TO THE OPENING EXCITATION?

It is classical that a tissue can be excited as the result either of the closing or the opening of a constant current. Normally the closing is so much more effective than the opening that if threshold currents are used, the latter effect does not enter. By using a dense anode, and a diffuse cathode however, or by killing the nerve under the cathode, the reverse may be the case, and a brief constant current now stimulates at the anode as the result of the cessation of the current. The strength-duration curve in this case has been investigated by Cardot and Laugier [1912], who found a "chronaxie" much longer than normal, and Prof. Lapicque was good enough to point out to me that the α curve might well be due to this phenomenon. He himself was investigating the question at the time of writing, and in a subsequent paper he has stated that the suggestion was not borne out by experiment

[Lapicque, L. and M., 1930], but since he does not mention what experiments he performed, it may not be superfluous here to describe a few which I made at the same time and which confirm his conclusion that the α excitation is brought about by the closing not the opening of the current.

My experiments were of six kinds, the first three showing that excitation occurred at the cathode, relying upon the validity of Pflüger's Law for its application to the present problem, and the second three showing directly that excitation was not the result of the cessation of the current.

(i) *The effect of temperature.*

The sartorius muscle of the frog was set up as shown in Fig. 3, upon the floor of a rectangular bakelite trough; a 1 cm. block was placed

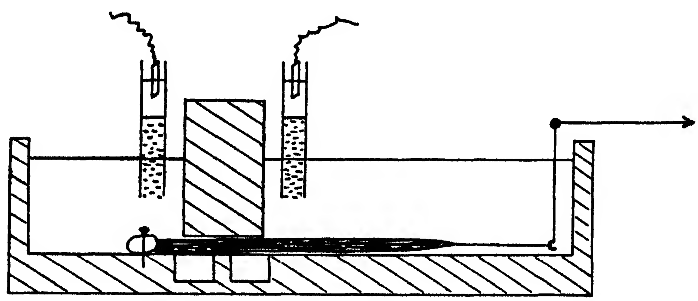

Fig. 3. Apparatus for warming and cooling anode and cathode with "block" electrodes. Note channels for hot and cold water in floor below block separated by mica sheet from fluid in trough.

above it near the pelvic end and non-polarizable (Zn-$ZnSO_4$-Agar-Ringer) electrodes arranged so that the current passed under the block, the cathode being near the pelvis. The floor of the trough below the block was cut away as shown to make two channels. These channels were completely separated from the interior of the trough, however, by very fine sheets of mica. In this way water at various temperatures could be passed through the channels without flowing into the trough, but the muscle, lying on the mica sheet, came intimately into contact (at the lower surface at least) with the temperature of the water. It was thus easy to warm or cool the anode or cathode independently and observe the effect upon the α curve.

The procedure was as follows. The muscle was set up and allowed to rest for an hour. The strength-duration curve was then taken at room

temperature (I). Then cold or hot water flowed below the cathode and anode as indicated below.

	Cathode	Anode
(II)	Cold	Cold
(III)	Cold	Hot
(IV)	Hot	Cold
(V)	Hot	Hot

Finally water at room temperature was passed through both channels (VI) as a control upon progressive changes. Fig. 4 shows the result of

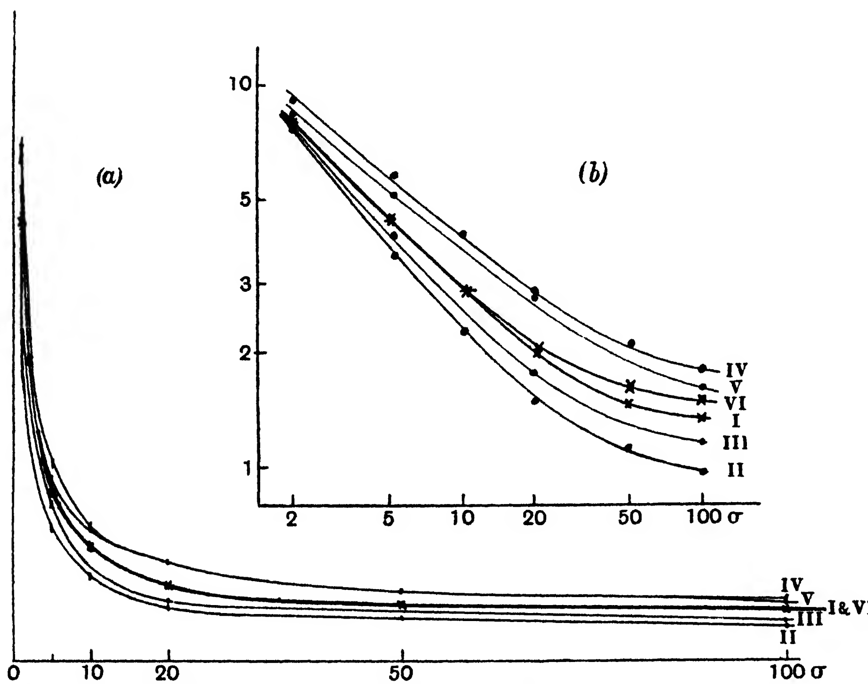

Fig. 4. (*a*) Strength-duration curves at various temperatures of anode and cathode (see text). Linear coordinates. (*b*) Curves of Fig. 4 *a* replotted in logarithmic coordinates.

an experiment where the room temperature was 10° C., and the cold and hot water in the channels was at 4° C. and 32° C., though the muscle probably did not attain these extremes. The complete set of observations took about 2 hours to accomplish, and at the end of that time the threshold had risen some 15 p.c.; to minimize the appearance of this progressive change in Fig. 4 it is assumed that the change occurred at constant rate, and each set of readings is reduced accordingly. Since

the temperature variations of threshold which concern us are greater than 100 p.c., the above assumption cannot in any case involve great error.

It is seen at once from Fig. 4 that when the cathode is cooled (curves II and III) the curve lies below that at room temperature (crosses), no matter whether the anode is hot or cold. Similarly when the cathode is warm (IV and V) the curve lies above, independent of the temperature of the anode. It is thus clear that the cathode is the region which characterizes the α threshold.

It may be wondered why threshold has been used above as criterion rather than excitation time. The latter measure involves an accurate knowledge of the rheobase, which in these α curves is an unreliable measurement, for the current has often to flow for a whole second or more before the contraction occurs, and the resulting polarization produces a change of threshold which is irreversible, or but slowly reversible, so that the rheobase is the least accurate of all points on the curve, and quite unsuited to take part in a characteristic constant.

Another application of temperature alteration to the present problem was as follows. The strength-duration curve for the sartorius was obtained with the same apparatus at room temperature, and then the current was reversed, and a second curve obtained. This second curve (as usual) showed a γ portion, but α was still prominent, and in one typical experiment the threshold lay on the α curve at a duration of 10σ. Thus at 20σ the threshold was undoubtedly α, whatever the direction of the current. The fluid was then withdrawn from the trough and a few drops of hot Ringer's fluid (40–50° C.) were dripped on to the muscle near the pelvic end of the block. Then the withdrawn fluid was replaced and the thresholds again determined for both directions of current at 20σ duration.

The results were as follows:

Initial threshold when cathode lay towards pelvis	14–13·5
,, anode ,,	17–16

After hot drops:

Threshold when cathode lay towards pelvis	20–19
,, anode ,,	18–17

It is clear that the treatment by heat caused no appreciable change in threshold when the anode lay towards the pelvis, but caused a marked decrease in excitability when the current flowed in the other direction. That is to say the excitability was affected by change at the cathode but not by change at the anode.

(ii) *The effect of electrotonus.*

The apparatus was that of Fig. 3, but in this case two additional electrodes were added. They were in the form of silver plates chlorinated and placed at a distance of 2 or 3 mm. apart close up to the block, both on one side or both on the other. When the Ag-AgCl electrodes were placed on the pelvic (cathodic) side of the block the polarizing current greatly affected the height of contraction resulting from an α stimulus sent in through the Zn-$ZnSO_4$ electrodes and localized under the block. The α stimulus was first adjusted to give a moderately small twitch when the polarizing current was not flowing. Then this current was turned on with the anode towards the block, and while the polarization continued the α stimulus was again sent in. The contraction now was abolished or much reduced. Reversal of the polarizing current on the contrary caused a considerable increase in the size of the twitch above the initial value. These results were not obtained, however, when the a n o d e of the α stimulus was polarized, for when the polarizing electrodes were placed on the tibial side of the block it was found that the polarizing current had no effect upon the threshold or height of contraction resulting from the α stimulus. We must therefore conclude again that the condition of the cathode is responsible for the α excitability.

(iii) *Measurement of conduction time.*

The apparatus used for this experiment is that described more fully later in this paper (Part III (*b*)). Suffice it here to say that the sartorius muscle was firmly clamped at the middle, and each end was attached to a lever writing on a fast-moving smoked drum. The usual block electrode was placed over the pelvic end and a duration of stimulus used which elicited an α contraction for either direction of current. Since the stimulus is applied in all cases to the pelvic third of the muscle the pelvic lever will move before the tibial one will, and this difference in latent period will obviously be more pronounced when excitation occurs at the pelvic end of the block than when the current is reversed and excitation occurs nearer the middle of the muscle.

A typical experiment gave the following results at a temperature of 20° C.:

	Difference of latent period
Cathode towards middle	15·8 σ
Repeated	16·3 σ
Cathode towards pelvis	25 σ
Repeated	26 σ

There is no question but that the latent period is greater when the *cathode* is the more eccentric, again showing that the α excitation arises from the cathode.

(iv) *A rheobase current.*

Turning now from the polar properties of the α excitation to direct temporal considerations, the simplest and perhaps the most convincing proof that the α excitation is not an opening excitation lies in the fact that the α strength-duration curve may be obtained as a smooth curve down to the rheobase, and the rheobase current certainly does not stimulate by an "opening excitation," for the stimulation is obtained

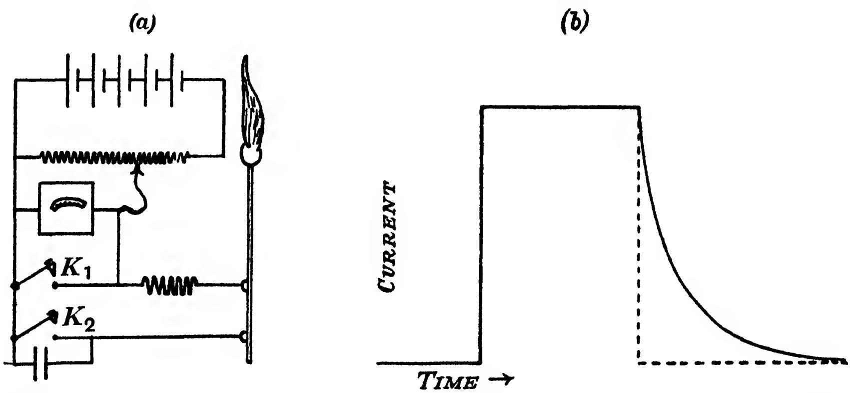

Fig. 5. (*a*) Circuit for stimulating with a current of the form shown in Fig. 5 *b*. (*b*) Rectangular current with exponential tail. Ordinates, intensity; abscissæ time.

equally when the current is allowed to flow on indefinitely. In fact I have never been able to obtain an opening excitation at all, since before the requisite intensity is reached the muscle goes into a prolonged closing tetanus which makes further increase of current valueless.

(v) *An exponential finish to the constant current*[1].

It is well recognized that the efficacy of a current of given intensity is very much reduced if it rise slowly rather than abruptly to its constant value. In the same way the efficacy of the opening excitation is greatly diminished if the current subside but gradually. By placing a condenser (as shown in Fig. 5 *a*)[2] across the second key of the usual strength-duration

[1] I am indebted to Dr Monnier of the Sorbonne (at this time passing through Philadelphia) for this elegant method.

[2] In a former publication [Rushton, 1932 *b*] Fig. 6 contains an error which, however, does not affect the text. K_2 should obviously be connected not to the extremity of the potentiometer, but to the slider, as in Fig. 5 (*a*) above.

apparatus, the current will not cease immediately upon opening K_2, but will decline exponentially as the condenser charges (Fig. 5 *b*). The effect of the condenser will clearly be twofold. In the first place it will cause a gradual decline instead of a sudden one, in the second it will cause an increase in the total quantity of electricity flowing.

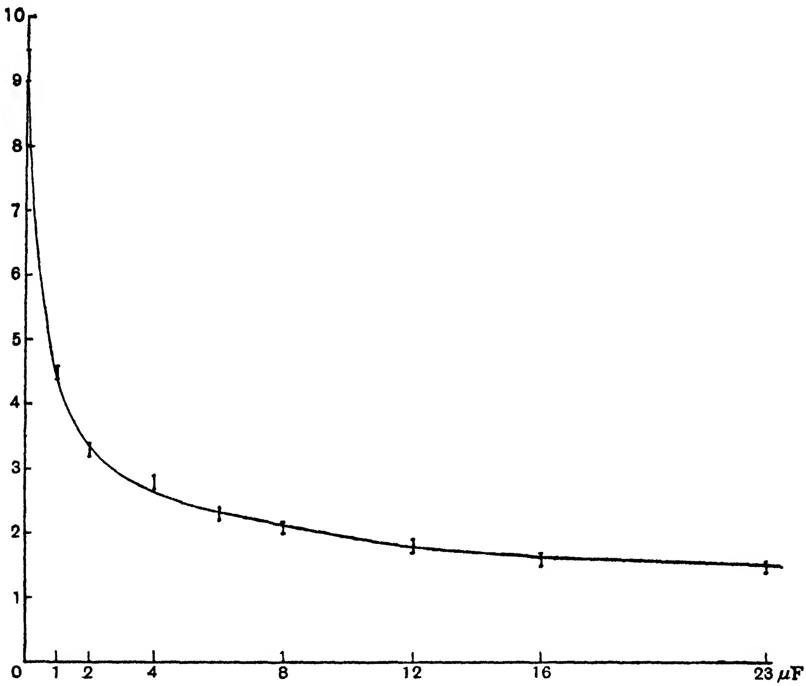

Fig. 6. Relation between strength (ordinates) and capacity in microfarads (abscissæ) for threshold stimulation by a current of the form shown in Fig. 5 *b*. Duration of constant current $= 5\sigma$, resistance through which condenser discharged $= 30{,}000$ ohms.

In view of the first it will cause an opening excitation to be less effective; in view of the second it will cause a closing excitation to be more effective. We may easily see whether in fact the condenser increases the efficacy or the reverse and hence learn whether the excitation is opening or closing.

From the experiment it at once appeared that the larger the capacity the more effective was the stimulus, showing that at all durations (and in this case especially the short ones) the α curve involves the closing excitation. Fig. 6 shows threshold plotted against capacity for a dura-

tion of constant current of 5σ; the lowering of threshold with increasing capacity is beyond question.

(vi) As a particular case of the foregoing we may consider the condition when there is zero duration of constant current, *i.e.* the ordinary voltage capacity curve obtained with condensers. As has already been published [Rushton, 1931] this curve resembles the α strength-duration curve in its long time relations as compared with nerve. There is no possibility that in this case we are concerned with the opening excitation since the threshold is lower when the stimulus declines more slowly, and thus those results confirm all the foregoing.

From the several kinds of evidence put forward we may certainly conclude with Lapicque that his earlier suggestion to account for the slow α time relation is not the case, and that the α excitation is elicited from the cathode by the processes set in operation at the start, not the cessation of a current.

PART III. IS THE α CONTRACTION PROPAGATED?

It is known that the direct excitation of muscle may give rise to two kinds of contraction, one propagated and the other localised at the cathode (Tiegel's contracture). Prof. Adrian was good enough to point out to me the possibility that the γ excitability might be correlated with the propagated contraction and the α with the contracture. This would obviously be a very satisfactory explanation of certain difficulties which are involved in reconciling the α phenomena with the views of Lapicque. The slow contracture would have a slow excitation time, the propagated contraction would be isochronous with the similar phenomenon in nerve. Unfortunately, however, this is not the solution of the difficulties for the α contraction is propagated. To show this I used two well-known methods, (*a*) by recording the thickening of the muscle at a point distant from the electrodes, (*b*) by clamping the muscle in the middle, stimulating in one half and noting the shortening of the other half. We proceed to a more detailed description.

(*a*) *Investigation of local thickening.*

The sartorius muscle was set up in a trough as usual and excited by the current concentrated below an ebonite block placed over the pelvic end of the muscle with the cathode towards the pelvis. The muscle was either attached by the tibial end to a light tension lever in the usual way (recording total shortening) or else it was looped loosely on the floor of

the trough, and over the tibial end rested a light small aluminium plate connected by a highly magnifying lever system to a pointer which would record by movement any thickening of the muscle under the little plate. In some experiments the muscle was tied down between the loop and the plate, to avoid any residual mechanical tug from the local con-

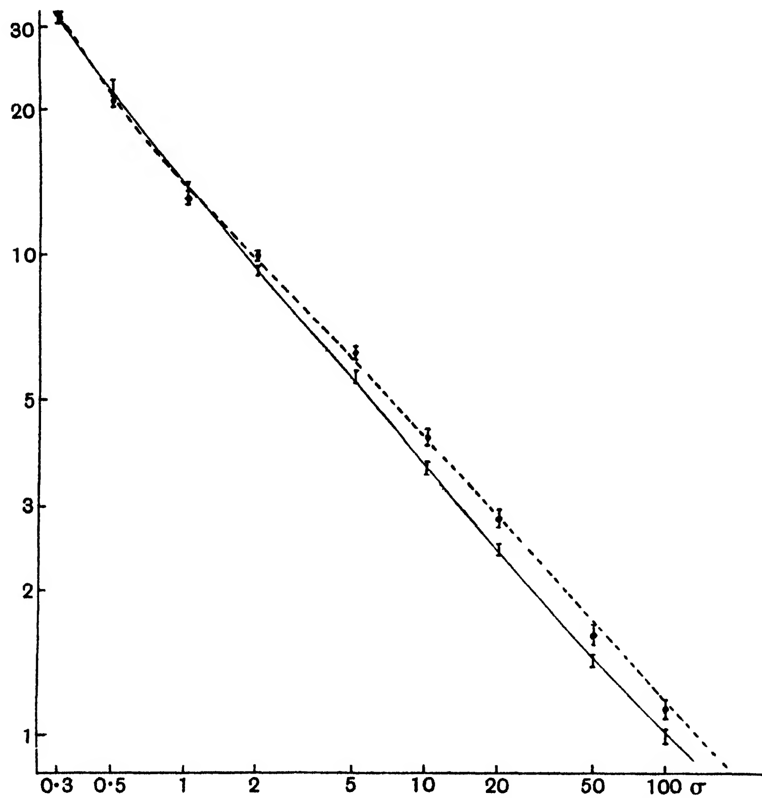

Fig. 7. Strength-duration curves of sartorius clamped in middle plotted logarithmically in both scales. Left of each pair of vertical lines represents threshold for pelvic contraction, right represents threshold for tibial contraction. Thresholds are identical throughout.

tracture, but the control indicated that there was no such tug. Strength-duration curves were obtained using as index either total shortening (continuous line, Fig. 7) or tibial thickening (intermittent line), and the results show clearly first that an α curve could be obtained equally by either method, second that the two curves were almost identical. The results are plotted in double logarithmic coordinates for clearness.

The slight divergence from identity in the two curves has no significance. The room temperature of 33–37° C. at this time made it necessary to conduct the experiment in a cooled box at about 15° C. The box had to be opened and the muscle trough withdrawn in order to change from one type of lever to the other, and thus a disturbance of temperature equilibrium as well as a difference of resting tension and exact localization of the cathode, all contributed to the slight difference observed in the two curves. These errors do not enter into the second type of experiment to be described, and here the two curves are absolutely identical.

(b) *Double lever experiment.*

A special trough was constructed which allowed the sartorius muscle to be clamped firmly at its middle, and to have both extremities attached to levers. I was not able to clamp the muscle rigidly without damaging some of the fibres, but a form of clamp which was found satisfactory was formed by a soft rubber strip in the floor, and above the muscle a hard rubber block about 3 mm. thick and as wide as the cross-section of the trough, having blunt teeth on the lower surface (Fig. 8). This was gently lowered on to the muscle until it gave a twitch, whereupon the block was clamped in that position. The muscle was attached to the two levers before clamping, so that the two halves of the muscle were at approximately the same tension. A 1 cm. block was placed over the pelvic portion of the muscle as usual and Ag-AgCl plates served as electrodes on either side of it, cathode towards pelvis, care being taken that neither block nor electrode touched the muscle. A strength-duration curve was then obtained using as index first the tibial then the pelvic lever, but in practice it was found that both levers moved together or not at all in nearly every case. Fig. 9 shows the results of one typical experiment, where the strength-duration curve is plotted in double logarithmic coordinates; each experimental "point" consists of two vertical lines representing the thresholds indicated by each lever. The experimental error (length of line) is seen to be about 5 p.c., and within those limits the threshold is identical for the two levers at all durations. There was practically no exception to this in any experiment, the greatest observed divergence being one of 10 p.c., where the threshold for tibial contraction was consistently higher by this amount. The explanation was no doubt that in this particular case the most excitable pelvic fibres were crushed at the clamp. If these results are accepted, therefore, it shows in a most convincing way that the α contraction is propagated.

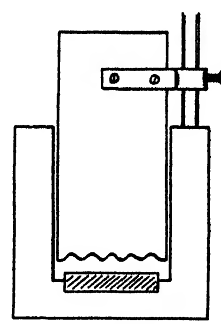

Fig. 8.

But the results cannot be accepted until we are satisfied that the movement of the tibial lever was not due just to passive pull under the clamp, and the real experiment consists in providing this evidence. In every case where the above determinations were made, two or more different controls were made as to the firmness of the clamp, and in no case was any slipping found to occur with tensions many times greater than those

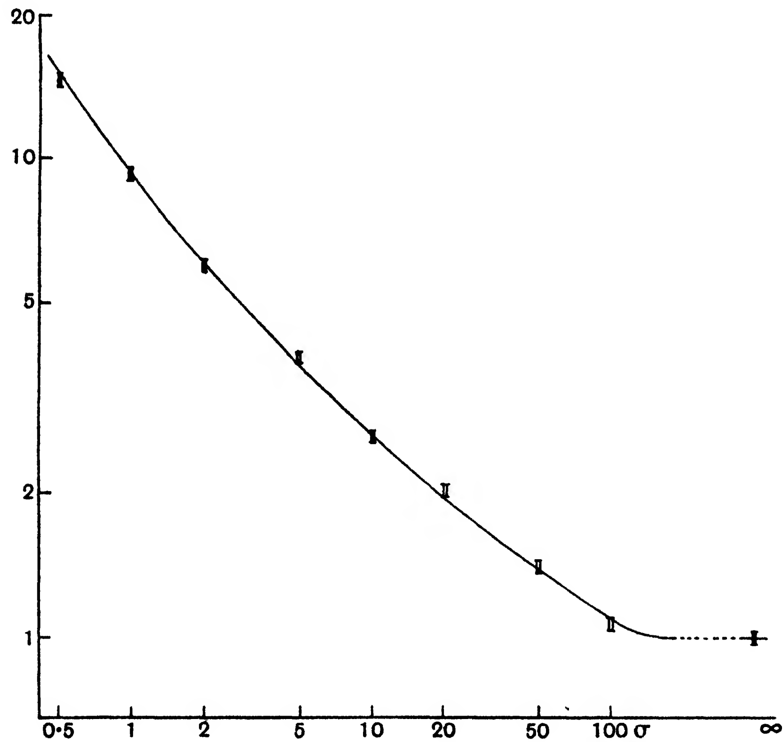

Fig. 9.

developed in these threshold measurements. The controls employed were as follows:

(*a*) Moving by hand the pelvic lever to far greater amplitudes than those actually involved, caused no movement of the tibial lever. The movements applied were sudden flicks to resemble the muscle twitch as far as possible.

(*b*) The muscle was killed with a hot iron applied along a transverse line on the tibial half not far from the clamp. A moderately strong

pelvic contraction now gave no movement of the tibial lever, though the increased rigidity of the killed portion would facilitate the propagation of a mechanical wave. It was finally ascertained that the opacity and swelling did not extend as far as the clamp, so that the muscle was not clamped more firmly by the process of killing.

Temperature coefficient of latent period difference.

The difference in the latent period of the two levers was due to the time occupied in the propagation of the contraction or of the passive tug from the pelvic to the tibial half. It would be expected that the temperature coefficient of propagation would be high, but that of a passive tug would be near unity. The latent period difference was easily measured from tracings on a fast-moving smoked drum, and from repetitions at various temperatures the temperature coefficient was obtained. Most of the observations fell in the region 15–25° C., in which range the Q_{10} lay between 1·5 and 2·3. The error is rather large, but even the lower limit is high for the effect of temperature upon a mechanical tug. Moreover, though flicking the pelvic lever causes no movement of the tibial one, yet often the contraction of the muscle causes equal excursions of the two levers, which is very difficult to explain unless the contraction is propagated.

Dependence of contraction upon local temperature.

If the two halves of the trough contain fluid at different temperatures, the rate of contraction of the pelvic half will be faster the higher the pelvic temperature. The rate of contraction of the tibial half on the other hand will be faster the higher the tibial temperature only if the contraction is propagated; clearly if it is due to a slipping at the clamp then it will be faster when the pelvic temperature is higher. The tracing (Fig. 10) shows clearly that the rate of contraction of the tibial lever is greater with higher local temperature but lower pelvic temperature, hence the contraction is propagated.

In view of these several controls, therefore, we may safely conclude that the muscle clamp was firm and that movement of the tibial lever signified a propagated contraction. This section therefore confirms and extends the results of section (*a*), and since in these experiments at least there was no appearance of Tiegel's contracture below the threshold for a propagated wave, it is clear that the α curve is not due to the use of this contracture as index of excitation.

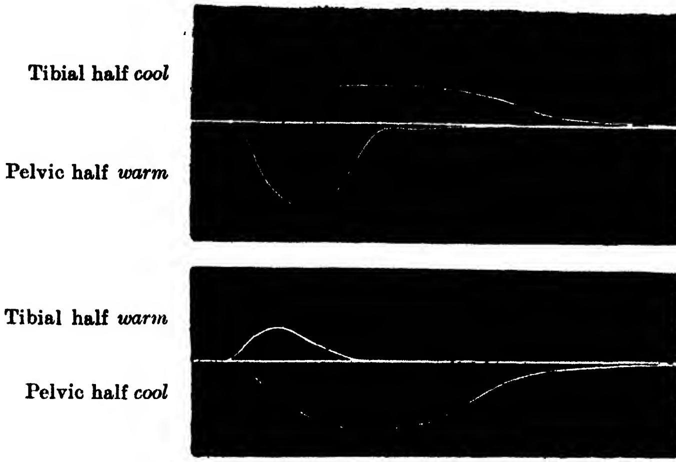

Fig. 10. Tracing of contraction of sartorius clamped in middle; read left to right. Each pair is a simultaneous record of contractions of two halves of muscle. Tibial above shortening upwards, pelvis below shortening downwards.

Part IV. Conclusions regarding α and γ excitabilities.

The literature of muscle excitation contains a considerable body of work upon the change of muscle chronaxie with drugs, fatigue, etc. In the majority of these cases it is not clear whether the tissue actually excited is muscle or nerve, or (more misleading still) whether the rheobase current excites muscle and one of twice this intensity at its minimum effective duration (*i.e.* chronaxie point) stimulates nerve, the chronaxie in this case being mainly dependent upon the relative accessibility of the two tissues to the stimulating current. This confusion, it appeared to me, was largely the result of using small "stigmatic" electrodes whereby the excitation time of muscle approaches that of nerve so that the two excitabilities are almost inevitably confused even when the whole strength-duration curve is plotted. In those experiments (unfortunately the majority) where the whole curve was not plotted but the determinations merely restricted to two points—the rheobase and double this intensity—the chronaxie measurement gives very little certain information. If observations have been such, for instance, that the authors have concluded that the muscle chronaxie has increased, it may be alternatively either that the nerve chronaxie has increased, or that the rheobase of the nerve has increased more than the muscle

rheobase has (or decreased less). But as this latter type of change is very likely to occur as the result of slight shifting of the electrode contacts, drying, etc., and especially if the tissue has to be removed, bathed, or manipulated, as is often essential for treatment with drugs, etc., these experiments require far more rigid controls than most accounts suggest have in fact been employed.

It was with the object of developing a technique to distinguish muscle from nerve, and rheobase change from chronaxie change that I undertook the investigation described in the series of papers [Rushton, 1930–2]. The essence of the difference between this method and that of Lapicque was the use of large fluid electrodes, which, as Lucas had shown [1907–8], give a long excitation time to muscle whilst leaving that due to nerve brief. Since Lucas's results had been questioned by Lapicque [1926] it was necessary first to confirm them in such a way as to meet Lapicque's criticisms. Next it was important to be able to obtain α and γ curves alone or combined as required, and finally to identify α and γ with muscle and nerve respectively in a more conclusive manner than had been done hitherto. Nearly all these experiments were made upon fresh surviving, undrugged muscles in what is generally considered good physiological condition. When for certain controls curarized muscles were used, it was left an open question as to whether the drug changed the chronaxie or not. The results of these experiments may therefore be applied without prejudice to future work upon the action of drugs and abnormal conditions.

Since the various phases of the argument are necessarily rather scattered in different numbers of this *Journal*, it may not be out of place to summarize here the principal steps.

(i) *The presence of two excitabilities was detected as follows:*

(*a*) The strength-duration curve when free from kinks was of one or other of two kinds, either γ with an excitation time the same as nerve, or α with a much longer excitation time [1930].

(*b*) When the strength-duration curve exhibited a kink, it consisted of γ at short durations and α at long durations, and the proportion of the two could be altered at will [1930].

(*c*) Threshold angle curves showed two excitabilities, one corresponding to fibres parallel to muscle and having α time relations, the other corresponding to fibres in quite a different direction (or directions) having γ time relations and being removed from activity by curare [1930, 1932 *c*].

(*d*) When the strength-duration curve appears to be nearly ex-

clusively γ, the appearance of α when present may be conclusively confirmed by latent period measurements [1931].

(*e*) In such cases also a double contraction arises from a constant current, the first due to γ excitation, the second to α more than 30σ later [1931].

(*f*) Kinks cannot be due to faulty contacts, etc. (cf. criticism by Hou [1931] of observations by Kodera [1928]), for in addition to the above evidence, results were obtained with four different pendulums, and with excitation by condenser discharges, and the kinks varied systematically with the position of the electrodes on the tissue [1930, 1931].

(ii) *The suggestion that α is due to some abnormal condition of the muscle appears to be incorrect on the following grounds:*

(*a*) An α curve of the usual form was present in spinal animals and in excised muscles at various times from excisions till 24 hours later [1930].

(*b*) The α substance is in physiological connection with its motor nerves [1932 *a*].

(*c*) The α curve may be elicited from all of a dozen different muscles investigated [1931].

(*d*) The α excitation is not due to the cessation of the exciting current (opening excitation) [*this paper*].

(*e*) The α contraction is not a Tiegel's contracture, but is propagated [*this paper*].

(*f*) It is not Bremer's neuromuscular contracture [*this paper*].

(iii) *The γ excitability obtained with fluid electrodes is due to the intramuscular nerves* [1932 *c*], *since:*

(*a*) The γ excitability has the same excitation time as nerve.

(*b*) The γ excitation time is like that of nerve nearly independent of electrode size.

(*c*) The γ substance is in the form of fibres.

(*d*) In the sartorius these fibres start their course at the exact place where the nerves enter.

(*e*) They run in this muscle towards tibia and towards pelvis for 8 mm. or more.

(*f*) In the sartorius they run in many directions, in the sterno-cutaneous strip they run in the direction of the nerve twig, and more or less perpendicular to the muscle.

(*g*) They are absent from the nerve-free pelvic extremity of the sartorius.

(*h*) When the strength-length curves from the sartorius show more than one excitable point, these always correspond to sharp bends in the nerves, and are closely correlated with nerve distribution despite the great variation from preparation to preparation.

(*i*) When the nerve is carefully removed by dissection from the surface of the sartorius, the γ curve initially very prominent disappears entirely from the cleared region.

(*j*) The action of curare upon the γ excitability is to abolish it completely by the time the indirect excitation (through the nerve) has failed.

(iv) *The α excitability is due to the muscle fibres themselves, since:*

(*a*) The α excitability is found in the pelvic (nerve-free) end of the sartorius, it persists after curare, and is due to fibres running in the direction of the muscle fibres (threshold angle results) [1930, 1932 *c*].

(*b*) The muscle fibres contracting to an α stimulus account for practically all the tension time developed by a twitch, and exhibit the same time tracing as a twitch [1932 *a*, and *this paper*].

In view of the experiments summarized above, the object of the investigation appears to be achieved, in that it is certainly possible by using fluid electrodes to obtain the strength-duration curve either of muscle or of intramuscular nerve fibres separately and without fear of confusion. This technique therefore avoids all those errors mentioned earlier which may invalidate the results when stigmatic electrodes are employed. Moreover the fluid electrode is particularly suited to the investigation of tissues in various conditions, for not only are errors due to drying eliminated, and variations in the exact contact of the electrodes excluded, but also these electrodes are better than most for investigations involving changes in the fluid bathing the muscle, since the fluid may be exchanged or circulated without affecting the contact between electrode and muscle.

An objection to the use of fluid electrodes, however, has been recently raised by Lapicque [1931 *a*, *b*], who claims that a strength-duration curve obtained in this way does not characterize the condition of the muscle as does the curve obtained when one of his forms of electrode is used. The original basis of this claim was that all truly characteristic curves must fit a certain empirical formula (Lapicque's Canon), but this argument loses its force when it appears [Rushton, 1932 *b*] that Lapicque's own method as applied to the frog's sciatic nerve does not satisfy the Canon, and that the divergence from the Canon which Lapicque's experiments show is real and not due to inductance errors, as he had supposed.

In a recent publication, however, Lapicque [1932] has put forward in a preliminary account, a theory to explain the "α effect," a correct understanding of which effect will naturally lead to the appreciation of the value and limitations of fluid electrodes. It will therefore be better to leave further discussion of this matter until a more complete development of Lapicque's theory has appeared.

In any case, whatever property of the muscle the α excitability characterizes, this method of excitation has the very considerable advantages that muscle is not confused with nerve, and the results are accurately reproducible.

Part V. A physical explanation of the "α phenomenon."

Perhaps the most interesting question which arises in connection with the α excitability is why the excitation time varies over so wide a range depending upon the type of electrode used. Now it may be shown that some variation of this kind necessarily follows from the physics of current distribution in muscle according to certain widely accepted ideas concerning excitation, but I had not intended to put forward this explanation before obtaining further proof of a quantitative nature. Since, however, an alternative hypothesis is being developed by Lapicque it seems of value to state here as briefly as possible the essential of the other for comparison.

The hypothesis I wish to put forward rests upon the following assumptions:

(*a*) A muscle fibre is a cylinder with a conducting core and a relatively resistant and polarizable sheath.

(*b*) Excitation results from the cathodal polarization of this sheath, which polarization must attain a critical value at some point.

(*c*) Ions are concentrated against the sheath owing to the applied electric field; the concentration is dissipated owing to the concentration gradient and potential gradient developed by the redistribution of ions.

If the current polarizing a large plane membrane is uniformly distributed over the whole of its extent (Fig. 11 *A*), then the motion of ions must be entirely perpendicular to the membrane (except near the edges where lateral diffusion may occur). If on the contrary the polarizing current is greater at one point (*P*) (Fig. 11 *B*) than others, the motion of ions will no longer be linear, for though the concentrating force is still along the normals to the membrane, yet, since the concentration at *P* is greater than at all other points in the neighbourhood,

diffusion will take place in a lateral as well as a normal direction. If now we imagine the plane rolled into a cylinder (with axis in the plane of the paper) so as to include the arrows, we obtain the condition of muscle excitation represented in the foregoing assumptions. The current passes through the sheath radially, and the above argument still holds. The conclusion is that if the muscle fibre is sufficiently long for the end corrections to be negligible, or if the ends of the cylinder are closed, then normal back diffusion alone will occur so long as the current leaving the sheath is the same at all points. If this polarizing current is localized to the neighbourhood of one point (by a stigmatic cathode for instance), then a lateral diffusion will be added to the normal back diffusion, and

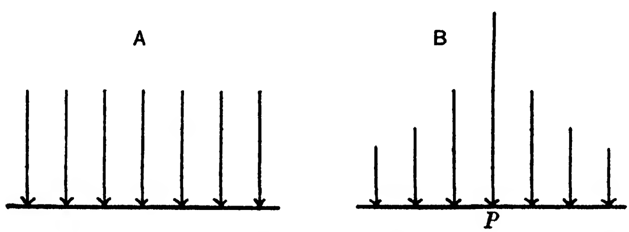

Fig. 11. Diagram of electric field forcing ions up against a membrane. Length of arrow represents magnitude of force at this point.

clearly this lateral diffusion will be more prominent the more localized the stimulus.

It remains to show that increase in lateral diffusion causes shortening of excitation time. For this argument it will be assumed that the amount of polarization developed by a given current in a fixed time is proportional to the intensity of the current.

This relation, introduced here as an assumption, follows as a necessary consequence of the application of linear differential equations to the excitation process. Since linear differential equations govern such phenomena as the flow of electricity in capacitative networks, continuous media, etc., and also the diffusion of particles and the propagation of waves, it would not be surprising to find that they also govern the excitation process which is supposed to depend upon these physical manifestations. In fact the theories of Nernst, Cremer, Hill, Ebbecke, etc., all express themselves in linear equations, and hence the above assumption may be deduced from each of them but is by no means limited to any one.

Let us consider two cases A and B (Fig. 12) where the current polarizing the membrane has the same maximum h in each case, but where the current falls off laterally much more rapidly in A than in B. Then the development of polarization at points A and B will depend

partly upon the polarizing current (the same for each at this point) and partly upon the normal and lateral diffusion. At the start, the concentration developed will be insignificant, hence the back diffusion will be negligible, and both polarizations will initially develop along the straight line OP, Fig. 13. But since lateral diffusion is much more pronounced in A than in B, the opposition to the linear increase in concentration will develop sooner and more markedly in case A, and curve A (Fig. 13) will diverge the sooner from OP and will reach the horizontal at a lower level than B. But since it is assumed that the amount of polarization produced is proportional to the intensity of the polarizing current, then

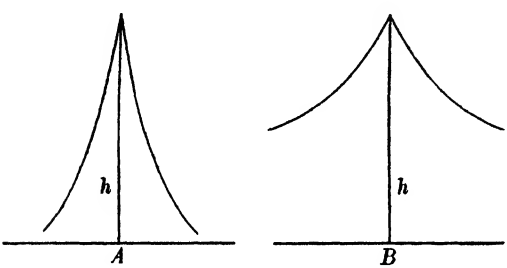

Fig. 12.

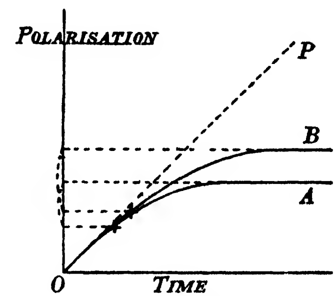

Fig. 13.

Fig. 12. Diagram of electric field forcing ions up against membrane. Ordinates of curves correspond to arrows in Fig. 11, *i.e.* to magnitude of force at various points along the membrane.

Fig. 13. Diagram of development of polarization in case A and B, Fig. 12. OP = development in absence of back diffusion. Crosses represent excitation times in two cases: greater lateral diffusion produces shorter excitation time.

the curves in Fig. 13 may be interpreted as follows. To produce a given threshold polarization, a long-lasting current (rheobase) must be higher in case A than in case B, but with currents of very short duration, the thresholds will be the same in the two cases. Hence A is relatively easier to excite at short durations, B at long durations. But it is known experimentally that tissues with short excitation times are those relatively easier to excite by brief currents, and *vice versa*; consequently, the greater the lateral diffusion, as in case A, the shorter the excitation time.

In view of these considerations we may conclude that the foregoing very generally accepted assumptions, without any new hypothesis, necessarily involve that the excitation time from stimulation with stigmatic electrodes will be less than from stimulation with large fluid electrodes. Whether the difference in the two cases is of the right order of magnitude

or not cannot be deduced from this qualitative analysis, but it seems worth while before postulating new conditions and mechanisms to see how far the foregoing concepts will take us.

It is interesting to note that lateral diffusion may explain also, qualitatively at least, three other well-known questions in nerve-muscle excitation.

(i) Nernst in his classical development of a Law of Excitation assumed that diffusion was only normal to the membrane. This we have seen is not in fact the case and lateral diffusion plays a very conspicuous part when stigmatic electrodes are employed (as in most of the experiments to test Nernst's Law). If Nernst's equation is solved for the two dimensional case

$$\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} = \frac{1}{\kappa}\frac{\partial c}{\partial t}$$

with boundary conditions similar to his, it is found that a strength-duration curve is obtained which is not asymptotic to the axis of durations but which has a finite rheobase, thus removing the most obvious and classical of the objections to Nernst's theory.

It is not unlikely that Nernst's neglect of the back E.M.F. of polarization was more serious than his neglect of lateral diffusion, but it is interesting to note that a finite rheobase may be deduced with no modification of Nernst's assumptions other than the obvious one that the cathode is more intensely polarized than other neighbouring points.

(ii) It has been known for many years that the excitation time of nerve depends upon the distance apart of the electrodes [Cardot and Laugier, 1914], and Lapicque [1931 *b*] recently has claimed that the same occurs with muscle excited through fluid electrodes. These observations are exactly what we should expect from the concept of lateral diffusion, for when the electrodes are close together there must obviously be a very rapid falling off in concentration from the critical exciting value at the cathode to zero at a point about half-way between the electrodes (Fig. 14), whereas the variation will be spread over a much greater distance with great interpolar lengths. Consequently in the former case there is a great lateral diffusion between anode and cathode which causes the excitation time to be shorter with shorter interpolar lengths.

(iii) The behaviour of nerve differs strikingly from that of muscle in regard to a change from stigmatic electrodes to fluid ones. Unless the present hypothesis is able to account equally for the comparative constancy of the nerve excitation time as for the great variability of the muscle excitation time, it obviously is inadequate. As a conducting

medium for electricity the medullated nerve differs from muscle most obviously in that it is surrounded by a thick insulating sheath pierced only at relatively long intervals by "faults"—the nodes of Ranvier. The obvious suggestion is that current enters the nerve principally through the nodes and stimulates there. But this suggestion, so evident on histological grounds, is exactly the condition to explain the different behaviour of muscle and nerve with various electrodes, for the lateral diffusion in nerve will almost entirely depend upon the size of the node and will be comparatively unaffected by the size of the electrode which leads the current to the outside of the node.

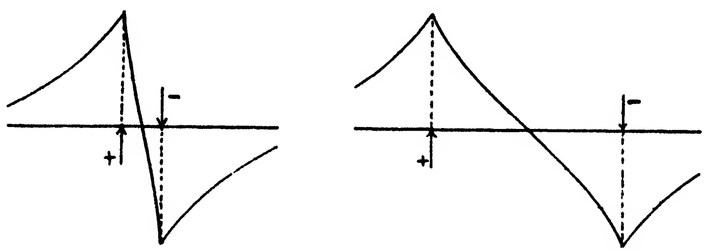

Fig. 14. Diagram of electric field forcing ions up against muscle membrane with electrodes near together or distant. Curves plotted as in Fig. 12.

In conclusion: the hypothesis which has been put forward fits qualitatively the facts of excitation of muscle and nerve with different kinds of electrodes, and this without having to postulate any new concept. It moreover admits of quantitative development to cover a very extensive range of excitability phenomena in a precise and fairly satisfactory manner (unpublished). It therefore appears that unnecessary complication may arise if an entirely new kind of excitation process be supposed to exist before evidence arises to show that the generally accepted process is inadequate.

Summary.

1. Reasons are given for concluding that the α excitability in muscle is the excitability of the normal muscle fibres themselves.

2. The isometric twitch is practically identical whether elicited by an α or γ stimulus. The rapidity of this α twitch excludes the possibility of identifying it with Bremer's neuro-muscular contracture which is very slow.

3. By several different methods the α excitation is shown to arise at the cathode due to the closing (not opening) of a current.

4. The α contraction is not a local Tiegel's contracture but is propagated.

5. The conclusions regarding the α and γ excitabilities derived from the present and former papers are tabulated.

6. Finally a hypothesis is put forward to explain qualitatively the dependence of muscle excitation time upon the nature of the electrodes and the relative independence of nerve.

It is a pleasure to express my thanks to Prof. Bronk, and my indebtedness to the Government Grants Committee of the Royal Society.

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THE ACTIONS OF ADRENALINE AND OF ACETYL-CHOLINE ON THE ISOLATED PULMONARY VESSELS AND AZYGOS VEIN OF THE DOG.

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THE work described in this paper was done at the request of Prof. I. de Burgh Daly, and was designed to accompany the investigations which have been, and are being, carried out in his laboratory on the pulmonary and bronchial vascular systems of the dog *in situ* [Berry, Brailsford and Daly, 1931; Berry and Daly, 1931; Daly and Euler, 1932].

PREVIOUS WORK.

The literature on the reaction of isolated pulmonary vessels to adrenaline begins with the work of Meyer [1906], who found that the drug contracted the extrapulmonary artery of the ox. Langendorff [1907] found contraction in most cases in experiments with extrapulmonary arteries from the sheep, pig, calf and cat. Neither of these two observers gave very full details of his work. Dixon and Halliburton [1910] found that the dog's pulmonary artery contracted with adrenaline for a distance of 1·5 cm. from its cardiac origin. The contraction became progressively less the farther away from the heart the sections were taken. Cow [1911] used the arteries of the sheep and ox for most of his experiments, but occasionally human, goat, or rabbit vessels. He does not state which species he used for the pulmonary vessels, but gives tracings from the rabbit and the sheep. He found that the extrapulmonary arteries showed progressively less reaction to adrenaline as they approached the lung, and that the intrapulmonary arteries were unresponsive. Campbell [1911] recorded contraction of the pulmonary artery of the sheep and of the rabbit, and, in seven out of ten cases, of the sheep's pulmonary vein. Barbour [1912] found an extremely strong contraction in two experiments with the main pulmonary artery of the rabbit. The extrapulmonary artery of the calf gave in four cases contraction, in one no reaction, and in two, where the sections were taken from the neighbourhood of the lungs, doubtful dilatation. In four cases

(three from the calf, one from the pig) intrapulmonary arteries showed no response. Macht [1914] used strips of the pulmonary arteries of pigs, oxen, and human beings, these strips being of various sizes and tested at various periods of time after death (49 days after death in one case). His concentrations of adrenaline varied between 1 : 1,000,000 and 1 : 1000. In every case the effect was contraction. Adrenaline produced no active dilatation after ergotoxine. Rothlin [1920] found that the extrapulmonary arteries of the ox and of the horse responded regularly by contraction. The rise to the maximum was rapid and the contraction was not long sustained. The intrapulmonary arteries also showed a contraction with 1 : 2,000,000 and 1 : 1,000,000, but it was not great. No qualitative change in reaction was produced by varying the concentration of adrenaline, and lengthening never occurred. Waterman [1930] worked with isolated veins of the dog and of the cat. In eight experiments he obtained contraction, and in the ninth a negative result. All the intrapulmonary branches he used gave a contraction. The greatest dilution of adrenaline was 1 : 70,000,000. Wissler finally [1931] recorded the results of sixty experiments with arteries from twenty-three oxen of varying age. He found that the regular response in the central vessels was a contraction. In some peripheral vessels definite dilatation resulted. Between these two areas lay an indifferent zone. The circumference of the vessels giving contraction was from 9 mm. upwards, that of the vessels giving dilatation from 11 mm. downwards. Contractions showed a rapid rise to their maximum, and usually disappeared within 5 min. The percentage shortening under optimum conditions was up to 5 per cent., whereas barium chloride caused a shortening of as much as 20 per cent. Some vessels responded to adrenaline 1 : 40,000,000, but the threshold was usually between 1 : 20,000,000 and 1 : 10,000,000. The reaction as a rule increased in degree with increase in concentration, but in some few cases, in which the threshold was high, there was no increase in degree. There was never any change in sign in the response with increase in concentration. The largest dilatation recorded was 7 per cent. Dilatations, in contra-distinction to contractions, were long-lasting, and also required in general higher concentrations of the drug (1 : 1,000,000) for their production.

The only experiments with acetylcholine on isolated pulmonary vessels are those of Waterman [1930]. He found no effect with acetylcholine in three experiments on pulmonary veins. On the other hand, pilocarpine in three cases gave vaso-constriction, and no effect in two others.

Method.

Nine dogs altogether were used, approximately a hundred experiments were performed, and about three times this number of applications of drugs were made. The dogs were killed with the humane killer of the slaughterhouse, so that there was no contamination of the vessels by anæsthetics. Ring preparations were used in all experiments (except one), and they were suspended in oxygenated Ringer's solution. The apparatus employed was an optical recording one, which is described elsewhere [Franklin, 1930], and the movements were magnified × 166 or × 160. The acetylcholine employed was the bromide (B.D.H.), and the adrenaline the ordinary 1 : 1000 solution (Parke, Davis and Co.), except in a few experiments in which, as a control, crystalline adrenaline (kindly supplied by the same firm) was used. The routine dilutions of acetylcholine used were 1 : 100,000,000, 1 : 10,000,000, and 1 : 1,000,000; some experiments were made with 1 : 10,000,000,000 and 1 : 1,000,000,000, but the effects were negative. The routine dilutions of adrenaline used were 1 : 10,000,000 and 1 : 1,000,000, but 1 : 100,000 was used in some cases when the tissue was old, and also in some of the reversal experiments. It was not possible to work with vessels much less than 4 mm. in circumference, hence the azygos vein was the only representative of the bronchial vascular system to be tested. The measurements of the vessels were made at room temperature and in general were somewhat less than in Ringer's solution at 37° C.; the relaxation on warming was greater in the case of the veins than of the arteries.

Results.

Adrenaline. The pulmonary aorta gave no effect once with 1 : 10,000,000, the extrapulmonary artery no effect twice with this concentration. Both vessels, in one instance each, gave a relaxation with adrenaline. In all other cases, *i.e.* in ten experiments with the aorta and twenty with the artery, the effect was a contraction. The highest degree of shortening was over 5 p.c. in the case of the aorta (Fig. 1), and over 12 p.c. in the case of the artery. The intrapulmonary arteries gave very small and variable responses with contraction predominating. The intrapulmonary veins also gave very small responses or none at all, and the relaxations balanced in number the contractions. The smaller extrapulmonary veins always contracted with adrenaline, the large extrapulmonary veins gave contraction or no response, never relaxation. The azygos vein responded by

contraction in eleven cases, by relaxation in two or three, and showed no effect in one or two.

The pulmonary aorta (Fig. 1), the extrapulmonary artery, large extrapulmonary vein (Fig. 2), and azygos vein (Fig. 3), all showed a

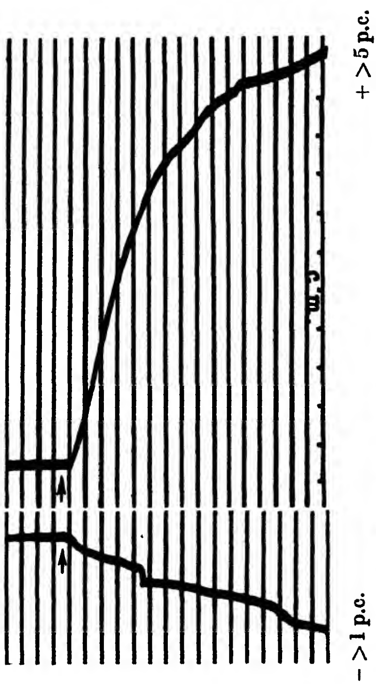

Fig. 1.

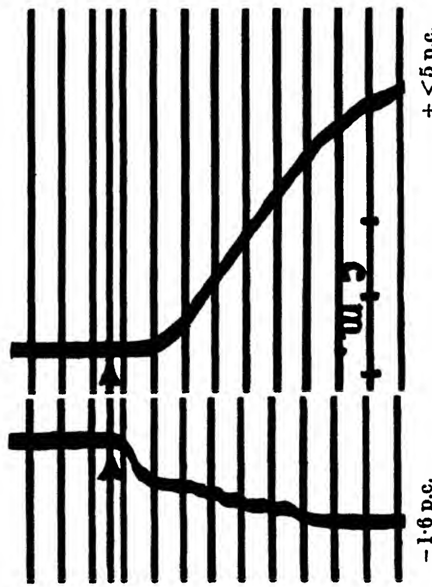

Fig. 2.

Fig. 1. Pulmonary aorta ring. Circumference 26 mm. Upper curve normal, lower after immersion of ring in ergotoxine ethanesulphonate solution. At arrows adrenaline hydrochloride 1 : 100,000. Time-grids minutes. Magnification × 160[1].

Fig. 2. Extrapulmonary vein ring. Circumference 9 mm. Upper curve normal, lower after immersion of ring in ergotoxine ethanesulphonate solution. At arrows adrenaline hydrochloride 1 : 100,000. Time-grids minutes. Magnification × 160.

reversal of adrenaline contraction after immersion in ergotoxine ethanesulphonate 1 : 50,000 or 1 : 20,000.

Acetylcholine. The pulmonary aorta once showed no effect with 1 : 100,000,000. In all other cases it gave relaxation. The extrapulmonary artery invariably relaxed (Fig. 4). The intrapulmonary arteries gave no

[1] Magnification in all figures refers to that shown in the *unreduced* records. The centimetre scales indicate the original size of the records before reduction.

response to the drug, while that of the intrapulmonary veins was variable, with contraction predominating. The smaller and larger extrapulmonary veins showed contraction (Fig. 5), which can be reversed by atropine sulphate in a concentration between one and ten times that of the acetylcholine (Fig. 6). The azygos vein never relaxed, and in most cases showed contraction, though in some the effect of the drug was negative.

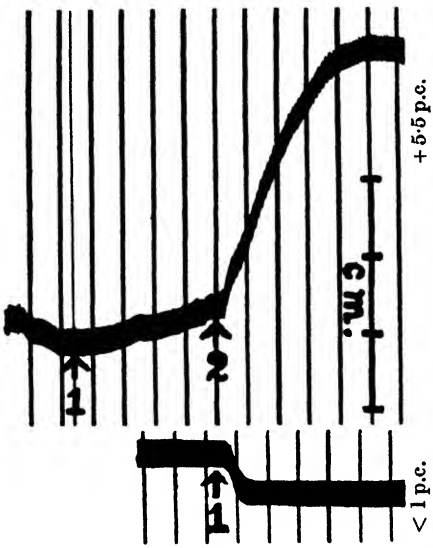

Fig 3.

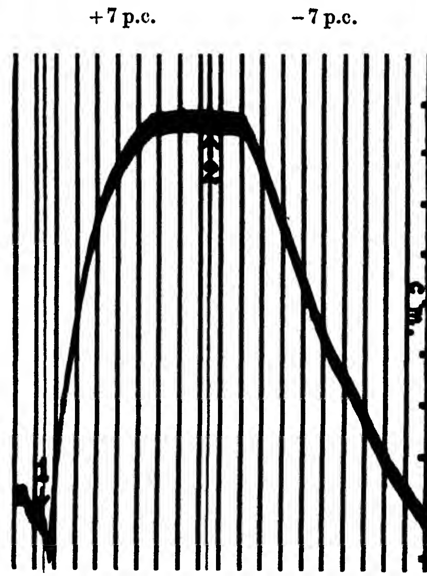

Fig. 4.

Fig. 3. Azygos vein ring. Circumference 8 mm. Upper curve normal, lower after immersion in ergotoxine ethanesulphonate solution. Upper curve (1) adrenaline hydrochloride 1 : 10,000,000, (2) 1 : 1,000,000; lower curve adrenaline hydrochloride 1 : 1,000,000. Time-grids minutes. Magnification × 166.

Fig. 4. Extrapulmonary artery ring. Circumference 16 mm. (1) Adrenaline hydrochloride 1 : 1,000,000, (2) Acetylcholine bromide 1 : 1,000,000. Time-grids minutes. Magnification × 160.

Discussion.

Taken on the whole, then, the extrapulmonary vessels of the pulmonary vascular system of the dog showed the following remarkable features. Both arteries and veins usually contracted with adrenaline, while the arteries relaxed and the veins contracted with acetylcholine.

Increase in concentration of either drug never changed the sign of an effect from contraction to relaxation or *vice versa*.

All parts of the extrapulmonary system, and also the azygos vein,

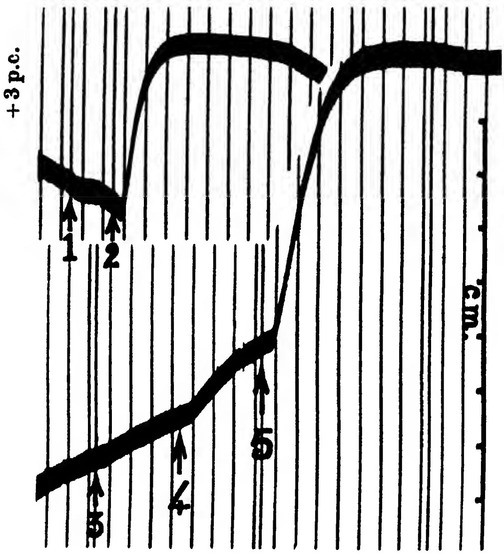

Fig. 5. Extrapulmonary vein ring. Circumference 8 mm. (3) Acetylcholine bromide 1 : 100,000,000, (4) 1 : 10,000,000, (5) 1 : 1,000,000. After replacement of Ringer's solution (1) adrenaline hydrochloride 1 : 10,000,000, (2) 1 : 1,000,000. Time-grids minutes. Magnification × 166.

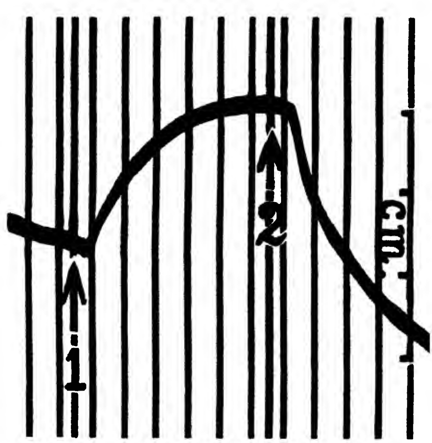

Fig. 6. Extrapulmonary vein. Circumference 10 mm. (1) Acetylcholine bromide 1 : 1,000,000, (2) Atropine sulphate 1 : 100,000. Time-grids minutes. Magnification × 160.

gave presumptive evidence, in the ergotoxine reversals of adrenaline contractions, of the presence of sympathetic vaso-dilator mechanisms in addition to the sympathetic vaso-constrictor ones. Control experiments with crystalline adrenaline proved that the reversals were not due to any other component of the stock 1 : 1000 solution (Parke, Davis and Co.).

In the single case in which a ring of pulmonary aorta relaxed with an initial dose of adrenaline, the piece was used 25 hours after the death of the animal. The isolated case in which relaxation occurred in a ring from the artery was similar, though it was from another dog. It was conceivable that in these instances the sympathetic vaso-dilators were naturally predominant over the constrictors, or that the lapse of time since death had affected the dilator endings less than it had the constrictor endings. The effect of lapse of time was therefore tested on the pulmonary aorta and artery from another dog, but after 50 hours the reaction to adrenaline was only changed in degree, and not in sign. This result, and Macht's instance of a contraction 49 days after death, incline one to the first alternative, and one may, in view of all the evidence, expect occasional dilator effects with adrenaline on isolated pulmonary vessels, as the literature tends to prove.

The azygos vein showed effects which were in general similar to those exhibited by the pulmonary veins, but there was a somewhat greater tendency to dilatation with adrenaline.

As adrenaline and, very probably, acetylcholine [Dale, 1929] are chemical agents produced in the normal body for the execution of definite functions in connection with the autonomic nervous system, it is important to consider if the concentrations of the two substances used in these experiments bear any adequate relation to their physiological concentrations. In this connection it may be stated that all the effects on the vessels described above have been obtained with 1 : 100,000,000 acetylcholine, and with 1 : 10,000,000 adrenaline, even if their degree has been increased with increase in concentration. Further, smooth muscle usually requires, in its isolated *in vitro* state, a higher concentration of a given drug than it does *in vivo*. It should also be noted [Hülse, 1922] that the pulmonary vascular system is more likely than the systemic to have a higher concentration of adrenaline in the blood passing through it. The final decision as to the validity of transferring the results of these experiments to one's conception of the vessels acting *in situ* must, however, be left until more precise knowledge exists of the concentrations and behaviour of adrenaline and of acetylcholine in the normal body.

At all events the amounts used in the present series compare favourably with those recorded in the literature.

One possibility, which is suggested by the action of acetylcholine on the extrapulmonary arteries and veins, is that congestion of the lungs, like asthma, may be due to excessive action of the parasympathetic system. If this is so, the neutralization of the venous effect (Fig. 6) by atropine points to a rational method of treatment. But more knowledge must first be obtained of the reactions of the intrapulmonary vessels, and one must also find out if human pulmonary vessels respond in a similar way to those of the dog. This latter point is to be investigated as soon as suitable material is obtained.

With regard to the intrapulmonary vessels, the writer's opinion is that they are much less reactive than the extrapulmonary ones, but that this point should be settled by the use of another technique (this is to be done by the aid of the microscope in Prof. Daly's laboratory). By comparison, however, with other small structures (*e.g.* trachea of fœtal kitten), the reactions of which have been recorded with the same apparatus as used in the present experiments, the small degree of the response of these vessels must be ascribed to weakness of innervation or scantiness of smooth muscle rather than to any fault of the apparatus.

In one case adrenaline was used on a longitudinal piece of pulmonary artery, instead of a ring, and it was found to cause contraction.

Summary.

1. In general isolated ring preparations of the extrapulmonary part of the pulmonary vascular system of the dog gave the following reactions with the concentrations used:

(*a*) Adrenaline constricted the arteries and the veins.

(*b*) Acetylcholine relaxed the arteries and constricted the veins.

(*c*) Adrenaline constrictor effects were reversed by ergotoxine.

2. Similar preparations of the azygos vein of the dog gave reactions similar, in large measure, to those given by the extrapulmonary veins.

3. The intrapulmonary vessels gave weak and variable reactions.

I wish to thank Prof. J. A. Gunn for the facilities afforded in his laboratory for carrying out this research, and for his interest in it. To Prof. I. de Burgh Daly I wish to express appreciation of his very generous cooperation throughout the investigation.

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THE FUNCTIONS OF THE GREAT SPLANCHNIC NERVES.

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"ON no subject in physiology do we meet with so many discrepancies of fact and opinion as in that of the physiology of the intestinal movements." This statement made by Bayliss and Starling in 1899 holds a lot of truth as applied to our knowledge of the subject at the present time. Nobody can state with precision what the functions of the great splanchnic nerve are in relation to intestinal or other activity. Contractile fibres to the spleen have just been demonstrated in this nerve by Barcroft and his associates [1932], and it is certain that its rôle in vascular and intestinal reactions needs further investigation and definition.

Having observed several irregularities of response while investigating with A. and B. Chauchard [1932] the variations of splanchnic chronaxie due to bleeding, etc., I determined to test the possibility of ascertaining something definite about splanchnic function by splitting the nerve into two or more bundles, so as to dissociate different activities in stimulating the separate branches. If one could approach the problem by artificially separating the different groups of fibres and stimulating the bundles individually and collectively some further light might be thrown upon it. Strict isolation of each strand of fibres corresponding to each function was not hoped for, but a preponderance of a certain function was expected to be exhibited by a given bundle.

METHOD.

The animals used in these experiments consisted of twelve dogs and four rabbits. They were always fully chloralosed. The great splanchnics were dissected out and cleaned on both sides. They were then cut as high as possible without injury to the diaphragm. The right nerve demands a good deal of care. Both nerves require to be cut in order to get good intestinal movement. The viscera were interfered with as little as possible. In some experiments both nerves were split longitudinally

into two parts, in others one of the nerves was split into two or three bundles. Separation into four parts was also tried and was found to be difficult; but it should be easier with improved technique. The rabbit's nerve was merely divided into two portions. The nerve in many instances was excited as a whole before division. The electrodes used consisted of a pair of silver wires across the lumen of a small vulcanite or glass tube about 2 in. long. On to these wires the nerve or bundle was drawn through a small opening at the lower end of the tube by means of a thread. The tube was then corked at the upper wide end, the stopper fixing the thread and holding the nerve in position. Thus the tube provided a practically water-tight chamber for the nerve. Excitation was effected both by galvanic and faradic currents, the strength varying from 4 to 6 volts in the former and the frequency from 5 or 6 to 12 per sec. Two pairs of electrodes were generally in place together, the exciting current being changed from one to the other by a switch.

The reactions observed were those of the intestine, of which the movements were recorded by the balloon method, and of the blood-pressure as taken from the carotid artery. Records from the artery were obtained either by means of a Hurthle's tambour with a mercurial manometer in the system, or by the mercurial manometer directly. The position of the balloon was varied; in many instances it was placed in the upper jejunum, in others at different lower levels. Two and three balloons have been used in some recent experiments, but the results of these are not at present included. The responses of the suprarenal gland and of the spleen could also be observed, but no means of measuring these were employed. Intense contraction of the exposed spleen could be seen as a result of splanchnic stimulation. The animal was not placed in a bath, but the viscera were well covered and kept at uniform temperature by warm saline irrigation.

Records.

Each record shown in this article is a double one of blood-pressure above and of intestinal movements below. The time represents intervals of 5 sec. On account of the occasional great excursions of intestinal records it was found simpler to get an assistant to mark with a cross the exact point of beginning stimulation than to use an electric signal. The duration of stimulation was 20 sec. unless otherwise stated. The blood-pressure was generally low with the splanchnics cut, namely from 65 to 80 mm. Hg. Figures for the different variations are not included as they can be pretty closely judged. All the records are from dogs.

Present views.

The older views concerning splanchnic function were modified as a result of the work of Bayliss and Starling [1899]. The conclusions of these authors have been questioned as regards certain details, but in the main they are still regarded as truths. They say that the splanchnic nerve exerts a tonic inhibitory influence on the intestine, that excitation of the nerve causes inhibition of the longitudinal and circular fibres, while there is no evidence that these nerves ever possess a motor function. The inhibition is independent of circulatory changes. The vagus, they say, possesses two sets of fibres, inhibitory and augmentor, the former having a short latent period and the latter a long one. This vagal action, they hold, is in no way due to spread to the splanchnic. Bunch [1899] said that he obtained evidence of intestinal vaso-dilator as well as vaso-constrictor fibres in the splanchnic nerve. On stimulation of the nerve he observed vaso-constriction at first and then in some cases vaso-dilatation. The vaso-dilatation he demonstrates by intestinal plethysmographic curves, and in none of his records is there any fall of arterial pressure from splanchnic stimulation. Bunch shows in one record a slight excitatory intestinal effect from splanchnic stimulation, but he makes nothing of it as demonstrating such fibres in the nerve.

Page May [1904] mentions that several observers believe that the splanchnics contain both motor and inhibitor fibres to the stomach; the vagus also is believed to have a similar double function. Openchowski [1899] and others also attribute a double function to the gastric nerves. Gastric innervation, however, which has recently been reviewed by McSwiney [1931], is a different consideration from intestinal innervation, though *prima facie* one may expect some similarity of action of sympathetic and parasympathetic supplies in both cases. It is stated by Weil [1931] that a reversal of the common effects ascribed to the splanchnic may occur, and Spadolini [1917] described a pure inhibitory effect from vagal stimulation and a pure augmentor one from the splanchnic, while opposite effects were given by different parts of the gut. Alvarez [1922] says that while the vagus generally has an excitatory and the sympathetic an inhibitory action on the gut these effects are transient and vary with strength of current, condition of the muscle, etc. This author says that vagus stimulation at first causes diminution of tone, and 15–60 sec. later increased activity. This corresponds in the main with the view of Bayliss and Starling, though Alvarez questions their conclusions on other points, such for instance as the characteristics

of the contractions. Bercowitz and Rogers [1921] have pointed out the importance of the rate as against intensity of stimulation in determining responses. This and other characteristics of excitability, chronaxie, and stimulation, are certainly important considerations. It looks as if a final solution of the problem will be reached only by thorough investigation of these features. The variations of response observed in the present experiments as a result of alterations of the frequency of the galvanic current were not sufficiently definite to be considered as distinguishing characteristics. The most effective frequency for stimulation was found to be 10 or 12 per sec., but the lower rates were not without effect. It is a point worth further investigation.

An augmentor function then is ascribed to the gastric sympathetic, but neither the authors mentioned nor others present any serious evidence to oppose the observation of Bayliss and Starling that splanchnic stimulation never gave intestinal motor effects. The chief point of the present communication being to show the occurrence of such intestinal motor effects of splanchnic excitation, I may anticipate the general statement of results by saying that an augmentor action on the contractions of the gut was frequently observed on stimulating different parts of the nerve. The variations of intestinal and vascular responses obtained on stimulating separate parts of the splanchnic nerve indicate the presence of different types of fibres in these nerves.

Results.

The usual effect obtained from stimulation of the whole splanchnic with a galvanic current was an increase of blood-pressure consisting of an immediate primary rise and a later secondary one. Inhibition of intestinal contractions usually accompanied the vascular effect (Fig. 1). This record shows the result of stimulating in A one half (the inner) and in B the other half of the left splanchnic nerve. Stimulation of the whole nerve previously had caused a response similar to B. In both cases there is a typical immediate inhibition of the intestine; the balloon was placed about 12 in. below the duodenum. The inhibition in A is followed by strong contraction and increase of the rate of activity; in B it is followed by increased rate only. In this experiment the application of the Faradic current caused a similar reaction in each case. In other instances a difference in the action of the two currents was observed. In neither of the reactions shown in Fig. 1 is there any appearance of a secondary or adrenaline inhibitory action on the intestine, though the

secondary increase of blood-pressure is obvious. This indirect effect of stimulation was well marked in other experiments.

The differences in the responses of different bundles of fibres were sometimes very pronounced. Much depended on the nature and duration of the stimulus, but uniformity of these factors was a condition applied for comparison of the separate parts of a nerve. One advantage of the method adopted is that excitation of different strands of fibres sometimes gives quite a good dissociation of intestinal and vascular reactions; a rise of blood-pressure given by exciting one bundle may not occur, or may be reversed, by exciting another, while the intestinal response remains unaltered. This is an important factor in deciding how much of an intestinal reaction may be due to the vascular change.

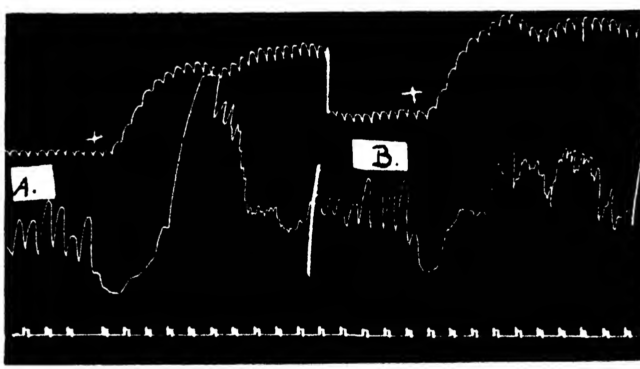

Fig. 1. Alternate stimulation of two bundles of left splanchnic. The effect on arterial pressure is the same, that on the intestine different. The same excitation was applied at + in each case. Duration 20 seconds.

In the foregoing experiment (Fig. 1) the increased activity of the gut might be said to be a natural reaction after the quiescent period, and more or less independent of nerve influence. But the augmentor effect was much more rapid in onset in other experiments. Stimulation of one-third of the right splanchnic for instance caused an almost immediate and marked increase of intestinal contractions (Fig. 2) with little or no vascular change. A minute or so later the effect had passed off, and a stronger stimulus at the second mark was applied to the same bundle. This time we have an initial period of inhibition lasting about 10 sec. and then a phase of pronounced increase of activity for about 30 sec. With this second gut reaction there occurs a definite fall of blood-pressure. The intestinal reactions following both excitations are very similar except

for the inhibitory phase in the second; the vascular reactions are not similar. That part of the intestinal response which is simultaneous with the change of blood-pressure is the inhibitory phase. It is not for this reason to be regarded as the consequence of the fall of blood-pressure. We get inhibition much more frequently with increase of blood-pressure. The excitatory phase also seems to be quite independent of vascular change, especially in the first reaction. In many other instances it was also quite unconnected with circulatory disturbance. Stimulation of the other thirds of the nerve under consideration caused increase of blood-pressure and inhibition of the gut.

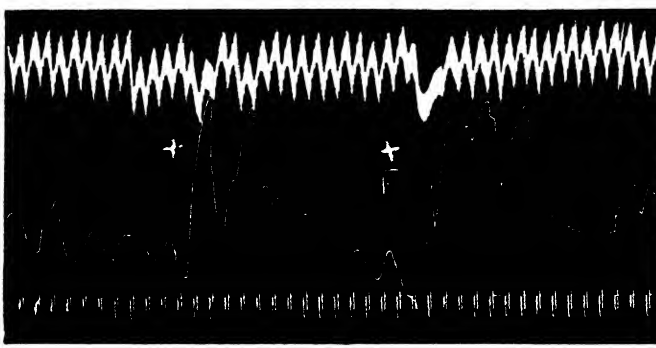

Fig. 2. Immediate increase of the gut contractions from stimulation (Faradic) of branch of right splanchnic. The second stimulus (stronger) applied to the same branch. A fall of blood-pressure occurs and again the contractions increase after initial loss of tone.

The right splanchnic nerve was separated into three branches in another dog. Stimulation of one of the branches caused intestinal inhibition lasting more than half a minute, followed by great increase of activity lasting more than a minute, all with practically no vascular changes (Fig. 3). This increased phase of activity is not presented as an example of an augmentor effect of stimulation, as it may perhaps be considered explicable as an after-effect brought about locally. The interval between the end of stimulation and the beginning of the marked increase of amplitude as compared with initial contractions is about 50 sec. As a latent period this interval is too long to permit one to describe the change as a simple reaction to the stimulation of the nerve.

Splanchnic bundles have been isolated stimulation of which occasioned pure inhibition of the intestine and pure hypotension in the

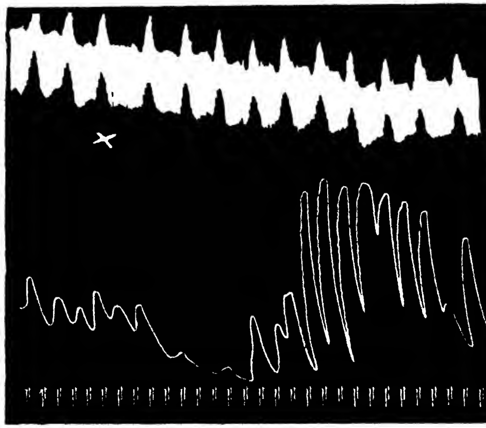

Fig. 3. To show intestinal inhibition from branch stimulation without blood-pressure change. The late increased activity of the gut to be compared with the quick response in Fig. 2. Stimulation 12 secs.

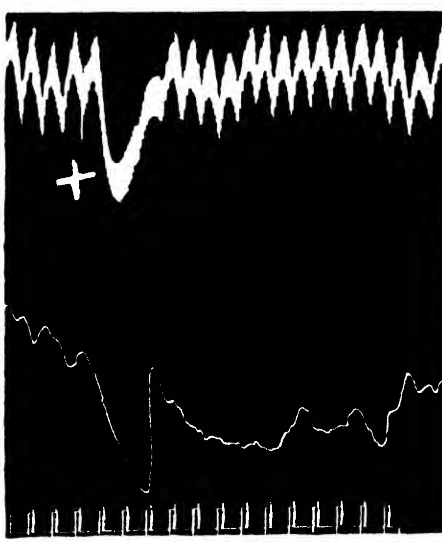

Fig. 4. Branch stimulation causing fall of blood-pressure and of intestinal tone. (Right splanchnic.)

circulation (Fig. 4). In this record no secondary increase of activity is shown by the intestine, rather a secondary loss of tone is seen which may be ascribed to adrenaline, though no influence of this is observed on the

pressure curve. Obviously there existed hypotensive and intestinal inhibitor fibres in the branch of nerve excited, and the stimulus (Faradic) was appropriate for both. Faradic stimulation of the other branch of this

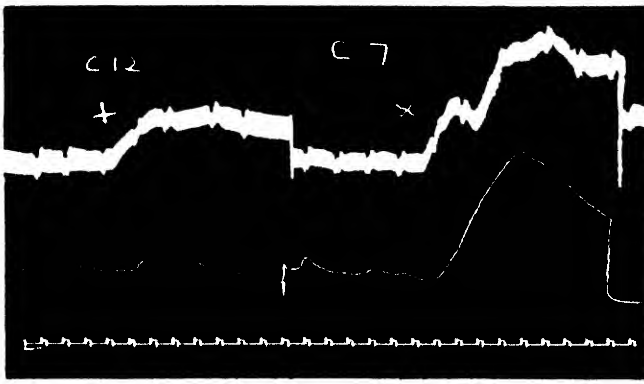

Fig. 5. Tonic effects of splanchnic stimulation; secondary coil at 12 cm. and at 7 cm.

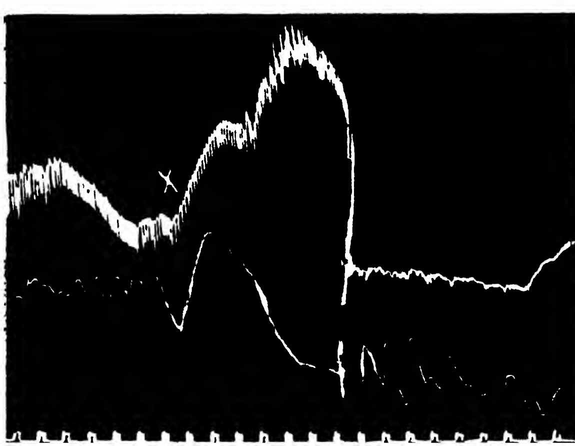

Fig. 6. Showing three phases of splanchnic influence on the intestine. Direct inhibition, increase of tone and adrenaline effect.

nerve caused a small rise of blood-pressure and inhibition of the gut. No augmentor intestinal effect was obtained, but that does not essentially mean that the nerve contained no augmentor fibres. In the first branch mentioned practically all forms of excitation elicited some inhibition but not hypertension.

When the natural movements of the gut are small there is frequently another type of augmentor reaction, namely a peristaltic or prolonged increase of tone (Fig. 5). The duration of the change in this record coincides with that of the blood-pressure where stimulation was strong. The secondary coil was at 12 cm. distance in the first excitation and at 7 cm. in the second. This peristaltic reaction may also exhibit a preliminary inhibitory phase (Fig. 6); here a falling off in tone occurs when the secondary increase of blood-pressure is pronounced. These variations all indicate much independence of intestinal activity in relation to vascular conditions.

A mixed reaction has also been observed, that is one in which a strong peristaltic contraction occurs while the regular or repeated contractions usually described as myogenic also become more active. This reaction, except for the preliminary period of inhibition, closely resembles the effect of acetylcholine on the gut in which intestinal excitation succeeds the hypotensive action after about 20 sec. The peristaltic form of response has also been observed with a fall of pressure. Several records of this effect have been taken.

Discussion.

The method adopted in these experiments is not altogether a satisfactory one for the dissociation of the functions of the great splanchnic nerves, but the results afford certain indications that these functions are more complex than is generally supposed. The method is one that may become more effective with improved technique and should provide a means of obtaining more definite information about these nerves and the characteristics of their excitability.

The existence in the splanchnic nerve of hypotensive fibres, vasodilator probably, is easily demonstrated. These are separate from the main group of vaso-constrictors. In one experiment strong Faradic stimulation (coil at 5 cm.) yielded a depressor effect, whereas a weaker current (coil at 8 cm.) led to a pressor effect. The galvanic current applied to the same branch of nerve always elicited a pressor effect. Apart from this instance the depressor fibres were found free from vaso-constrictors and responded to different strengths of current. These depressor fibres do not seem to occupy a large volume of the nerve trunk. No precision could be achieved in determining their orientation, a statement which also applies to the other groups of fibres. Vaso-constrictor effects were much the more common in the excitation of the different artificial

bundles and seemed indeed to indicate that vaso-constrictor fibres were more abundant and more susceptible to ordinary stimuli than the others.

With regard to intestinal inhibitory fibres we have to distinguish between a direct primary action and a late effect due to the increased absorption of adrenaline. The initial inhibition was freely obtained on stimulating different bundles and it was frequently followed by an augmentor effect. But this primary inhibition was absent in some clear instances and no form of stimulation of the whole nerve could elicit it. It was not always simply a case of missing the inhibitory fibres going to a particular region of the gut. Sometimes a difference was observed as between right and left nerves, excitation of one causing inhibition while that of the other did not. This may mean a different distribution to the gut.

The augmentor action on the gut, already referred to, is distinctly twofold in character, viz. a slow tonic effect and an increase of rapid contractions. A quiescent condition of the intestine is rare after section of both splanchnics. It was well marked in one animal in gestation of about the fifth or sixth week. In this experiment the predominant reaction from both nerves was the slow peristaltic contraction which was accompanied by increase of blood-pressure. The augmentor reaction in general reminded one of vagal effects and set one thinking of such a possibility. None of the authorities on vagal distribution mentions a possible junction of sympathetic and parasympathetic fibres in the splanchnic nerves. McCrea [1924] says that these two sets of fibres join together on their way to the liver. This writer, McSwiney and others, whom the latter quotes, also describe such union on the mesenteric and pancreatic arteries. Bayliss and Starling make no reference to such a possibility as splanchnic and vagal union. I have discussed the matter with some prominent anatomists. One of these says that he would not be surprised to learn that a blend of vagal and splanchnic fibres occurred at diaphragmatic level.

Atropine in large doses failed to abolish the augmentor reaction in these experiments, but, as Bayliss and Starling point out, atropine does not paralyse the intestinal vagus. Ergotoxine modified the response but did not cause its abolition; further tests with sympathetic paralysants are necessary.

Vagal stimulation proper yielded gut reactions very similar to those obtained with splanchnic stimulation, the preliminary inhibition in the latter case being a particular feature in some instances. The action of acetylcholine is of interest; in four or five experiments in which it was

injected it showed no inhibition, but otherwise closely resembled certain effects of splanchnic excitation. All that can be said at present is that these augmentor fibres which undoubtedly exist in the great splanchnic nerves exhibit a vagal type of reaction, but it is possible that they belong to the sympathetic system.

SUMMARY.

A method was adopted for investigating the functions of the great splanchnic nerve in the dog and rabbit by splitting the trunk longitudinally into two or three branches and stimulating each separately.

Stimulation of a branch sometimes caused a fall of blood-pressure, accompanied by inhibition, augmentation or no change of intestinal movements.

Branch stimulation sometimes occasioned marked increased activity of intestinal movements with or without preliminary inhibition. This reaction is similar to that given by stimulation of the vagus nerve, but there is no evidence that the parasympathetic system is in play.

The results as stated have been chiefly found in the dog, not satisfactorily in the rabbit. In the former animal the great splanchnic nerve contains hypotensive vascular fibres and augmentor intestinal fibres, which with the generally admitted fibres of opposite function in each case constitute two reciprocal systems.

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PROCEEDINGS

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A pleural cannula. By K. J. FRANKLIN and H. P. GILDING[1].

The cannula consists of a metal tube, into which is screwed a bullet-shaped head. The head is steel-tipped, and a washer is interposed between it and the tube. The tube is shown in section in Fig. 1. Between *d* and *e* the upper half is cut away to make a communication with the thoracic cavity. Between *b* and *g* the metal has been turned down, so that the tube has a slightly smaller external calibre between these points. At *c* and *f* are small grooves which run round the tube. At *a* and *h*, on the upper surface of the tube and equidistant from the central hole, are two small drill holes. The diagram shows a size of cannula suitable for use in rabbits and cats. It is passed across the thorax in the supine position of the animal through corresponding intercostal spaces on the two sides.

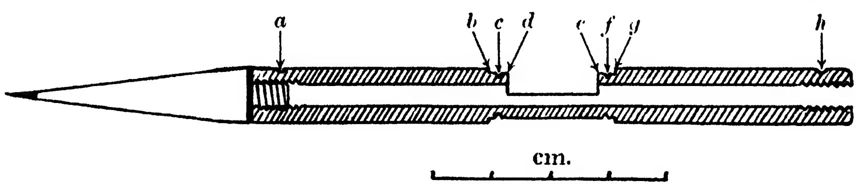

Fig. 1.

When passed, it should lie over the heart and a little below the sternum. The depressions *a* and *h* should be uppermost, and equidistant from the mid-line of the animal.

The cannula depicted is used for another purpose, and will be dealt with later by one of us (K. J. F.). For use as a pleural cannula only, the bullet-shaped head might be turned on the same piece of metal and not screwed to the body as in the diagram, similarly the threaded bore at the tail end is unnecessary for this purpose.

For getting an idea of the degree of constriction or relaxation of the bronchi in a decapitate animal under the action of various drugs, the apparatus is connected with a suitable recording apparatus. For measuring the intrapleural pressure variations in an intact animal, a portion

[1] Assisted by a part-time research grant from the Medical Research Council.

of condom is fastened between *b* and *g*, by threads passing round the grooves *c* and *f*, before the cannula is inserted. The slightly smaller calibre of the tube between *b* and *g* prevents any tearing off of the membrane during insertion. The cannula is filled with fluid and connected with a recording manometer, and during insertion, which takes only a second, a negative pressure within it ensures the collapse of the rubber membrane. The bullet-head prevents any occurrence of pneumothorax on passage of the cannula, and, gripped as the tube is between two ribs on each side,

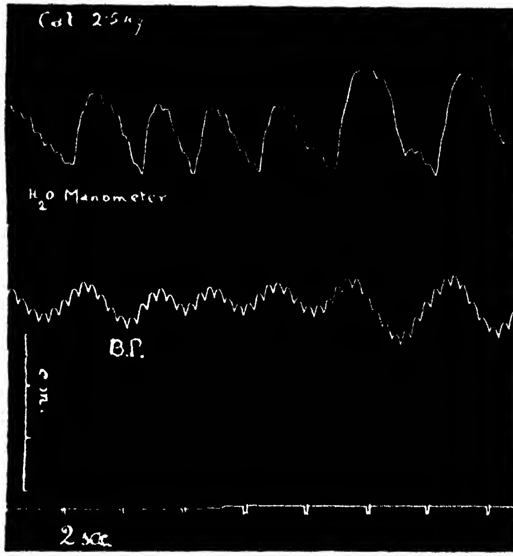

Fig. 2. Cat 2·5 kg. Upper tracing: H_2O manometer.
Lower tracing: blood-pressure.

it is unaffected by movements of the animal. In addition, as it has only one hole within the thorax, and this is turned to face the sternum, no blocking can occur as the result of movements of the thoracic viscera. If the sternal region is pinched up in an intact animal, or the artificial respiration temporarily reduced in a decapitate animal, there is no danger of the bullet-point puncturing the lungs during the passage of the cannula. Fig. 2 shows a record of pleural pressure obtained with the cannula connected to a 6 mm. bore water manometer, the writing-point being attached to a glass float.

The CO_2 catalyst present in blood. By R. Brinkman, R. Margaria, N. U. Meldrum and F. J. W. Roughton.

A year ago Brinkman and Margaria showed that minute traces of hæmoglobin, whether purified or crude, accelerated strongly the hydration and dehydration of CO_2 (*i.e.* the reactions $H_2CO_3 \rightleftharpoons H_2O + CO_2$ and/or $\overline{HCO_3} \rightleftharpoons CO_2 + \overline{OH}$). Their technique consisted in registering the pH changes in a thin film of $NaHCO_3$ solution adhering to an antimony electrode, when air containing 10 p.c. CO_2 (for the hydration velocity) or no CO_2 (for the dehydration velocity) was passed over the film. Many new results have since been obtained by this technique, but these have all proved to have only a qualitative significance, since controls on the reactions in absence of catalyst showed that the changes observed were, for some reasons not yet explained, far too slow. We have therefore confirmed and extended the work by other methods, the validity of which have been checked by experiments on the uncatalysed reaction.

A special glass vessel, containing two compartments, was mounted in a shaker enclosed in a thermostat, and was connected to a manometer of the Barcroft type. In one compartment 2 c.c. of 0·1 M/$NaHCO_3$ were placed, in the other 2 c.c. of 0·15 M buffer pH 6·4–6·6. Cacodylate and phosphate buffers gave similar results. On starting the shaker, the solutions mixed within 2–4 seconds and the subsequent evolution of CO_2 during the shaking was registered by the manometer readings over a period of usually 4 minutes. The catalytic effect of various solutions was tested by adding 0·1 c.c. to one or other of the compartments before the shaking.

The reaction rate was doubled by 0·0012 p.c. purified HbO_2(Ox). Higher concentrations of HbO_2 up to 0·01 p.c. (the highest tried) gave proportional increases in rate. Reduced hæmoglobin and methæmoglobin both gave the same rate as HbO_2; CO hæmoglobin on one occasion gave the same rate, but on another a slightly faster rate. Lysed corpuscles of the same concentrations in hæmoglobin also gave about the same rate, but unlysed corpuscles were two to three times less effective, owing partly no doubt to slowness of diffusion of substrate into the corpuscle. Serum and plasma were both negative. Of the breakdown products of hæmoglobin, hæmin was negative, but globin, prepared by Anson and Mirsky's method and also by Hill's, gave acceleration roughly equal to that by hæmoglobin containing the same amount of globin. So also did hæmoglobin which had been resynthesized from globin and hæmin. Crude muscle hæmoglobin, prepared by perfusion of the hind limbs of a dog or cat with Ringer till the perfusate was colourless, followed by extraction

of the muscles with water, had about the same activity as the blood hæmoglobin of the same animal.

The blood pigments of a number of lower animals were also tested. Of Hb-containing animals the blood of frog, gold-fish, *Chironomus*, *Arenicola* gave marked effects, but less than those of mammalian blood, whereas earth-worm and planorbis blood appeared to be quite inactive.

The chlorocruorin of *Branchiomma* was also quite active.

The hæmocyanin of *Maia* and snail seemed to give a slight positive effect, but this requires confirmation.

The action of 0·001 cyanide on mammalian Hb has so far proved perplexing. In some cases it has enhanced the rate by about 40 p.c., whereas in others it has caused complete inhibition.

Some of the above results have also been confirmed by photoelectric recording of the pH changes of mixtures of bicarbonate or carbonic acid after being mixed with suitable buffers containing suitable indicators.

This catalytic power of blood has proved to be of use in analytical chemistry, for it facilitates greatly the titration of carbonate in mixtures either of NaOH and Na_2CO_3 or of Na_2CO_3 and $NaHCO_3$.

Addendum by N. U. Meldrum and F. J. W. Roughton.

Recently we have succeeded in separating the catalytic system from hæmoglobin, as shown by the following experiments:

(*a*) When HbO_2(Ox), purified by Adair's method, was precipitated by contact with $CHCl_3$ for 36 hours at room temperature, the filtrate, although free from Hb and globin, was found to have almost the same activity as the original solution.

(*b*) Charcoal adsorbed the active system, for solutions of HbO_2 filtered after shaking with charcoal, showed only a fraction of the initial activity.

(*c*) When 1 °/₀ HbO_2(Ox), pH 6·8 was warmed to 55° C. for 35 minutes the activity was destroyed, although the hæmoglobin was still mainly methæmoglobin, which is normally as active as HbO_2.

For this new enzyme system we suggest the name Carbonic Anhydrase.

The influence of iodoacetic acid on the blood sugar level.

By J. T. Irving. (*Dept. of Physiol., Univ. of Bristol.*)

In confirmation of the previous work of Neuss [1931], I have found that subcutaneous injections of sodium iodoacetate into rabbits produced a marked hyperglycæmia. The dose used varied from 10 to 20 mg. per

100 g. body weight (calculated as free acid). Immediately on injection, the blood sugar began to rise and the typical symptoms came on. Death usually occurred in from ½ to 1½ hours. About half the rabbits showed extreme rigor at death, but the appearance of rigor was commoner in those which had died quickly. In all cases but one the blood sugar rose steadily till the animal died, typical figures at death varying between 0·2 and as much as 0·5 p.c. The liver glycogen values at death were found to be very variable, but nearly all indicated that the glycogen reserves were by no means depleted. Iodoacetic acid was found not to interfere with the estimation of the blood sugar.

After the production of insulin hypoglycæmia, the injection of iodoacetate immediately caused the blood sugar to rise, and convulsions to cease, but iodoacetic-poisoning symptoms soon supervened, and the animals usually died in about half an hour, often showing marked rigor. The intravenous injection of insulin after iodoacetic acid given subcutaneously had no effect on the blood sugar values or the onset of symptoms.

Experiments on isolated organs have not yet been made, so it is impossible to say to what extent the rise in blood sugar is due to stimulation of the liver, or to inhibition of glycolysis in all the tissues. In view of the invariably fatal issue, the latter factor must play an important part, in which case it appears possible that the first change which the blood sugar undergoes on reaching the tissues is a degradation to lactic acid.

The expenses of this work have been defrayed from a grant from the Government Grants Committee of the Royal Society.

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The pituitary and the reactivity of the uterine muscle.

By J. M. Robson. (*Macaulay Laboratory, Institute of Animal Genetics, Univ. of Edinburgh.*)

Previous experiments have shown that the intramuscular injection of bovine anterior pituitary lobe substance (together with an antiseptic, quinanil) into œstrous rabbits is followed by the formation, in the ovaries, of luteal tissue, whose period of activity is equivalent to that of normal pseudopregnancy; progestational proliferation of the endometrium and inhibition of the *in vitro* reaction of the uterine muscle to pituitrin are observed for 14–15 days after a single injection [Robson, 1932]. The

regular injection of one anterior lobe daily causes marked luteinization of the ovaries; the uterus shows the usual progestational proliferation, but inhibition of the pituitrin reaction is no longer observed by the 7–8th day after the first injection: on the contrary, pituitrin now causes contraction of the muscle. The disappearance of inhibition in the presence of proliferation represents a "dissociation" of the responses of the uterus. Doses of an extract of the corpus luteum which cause inhibition of the pituitrin response in test animals do not produce inhibition in animals in which "dissociation" has developed (even though the injection of anterior pituitary lobe substance be continued).

The daily injection of both anterior pituitary and α-hormone (œstrin —about 10 M.U. of an oily solution daily) into intact œstrous animals caused progestational proliferation and inhibition of the pituitrin reaction in the majority of experiments; in a few experiments, however, the dissociation of the uterine responses was still observed, although the histological appearance of the uterus showed that more than adequate doses of œstrin had been given. The daily intravenous injection of α-free gonadotropic preparations made from the urine of pregnant women [Wiesner and Marshall, 1931] brought about progestational proliferation and inhibition of the pituitrin response. Both reactions were maintained for prolonged periods and no "dissociation" was ever observed under these experimental conditions.

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May 14, 1932.

Myotatic reflexes during sympathetic stimulation. By GILBERT PHILLIPS[1]. (*Univ. Sydney.*) *Physiological Laboratory, Oxford.*

In an article now in the Press [1932] changes in myotatic reflexes following the sympathetic denervation of skeletal muscle have been described. Proprioceptive reflexes have now been examined in the soleus muscles of decerebrate cats during faradic stimulation of one sympathetic chain in the abdomen[2]. Using dissecting binoculars and a headlamp the sympathetic chain on one side has been carefully freed over a length extending caudally from the crus of the diaphragm for 2·5 to 3·5 cm. The rami of the first and second lumbar sympathetic ganglia have been cut, and glass shielded electrodes placed on the isolated sympathetic chain.

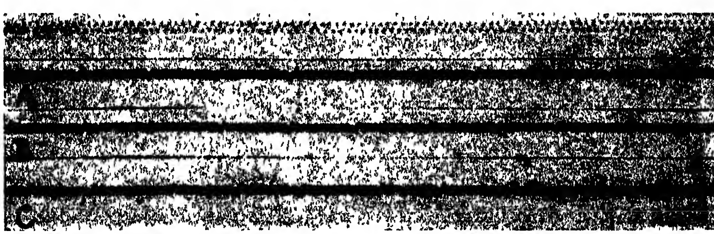

Fig. 1. Galvanometric record of firing of single muscle fibre unit under slight constant stretch. A, normal resting discharge. B, after one minute of sympathetic stimulation. C, after two minutes of sympathetic stimulation. A second unstable unit fires somewhat irregularly in the background. Tuning fork 100 D.V. per second.

The stimulating circuit has consisted of an inductorium having 2 amps. in the primary circuit with a coreless secondary coil at a distance of 5 cm.

To avoid polarization over long stimulation periods both make and break shocks delivered at 10 per second by silver chloride coated electrodes have been used.

[1] Fellow of the Rockefeller Foundation.

[2] Dr Ragnar Granit assisted in this work.

It may be justifiably assumed that under such stimulation all structures in the soleus muscle innervated by post-ganglionic sympathetic axones were excited, since all post-ganglionic fibres were intact from the sympathetic chain to the periphery; the rate and strength of stimulation were adequate [1929]; and the amount of operative handling was insufficient to interrupt the activity of sympathetic neurones [Adrian, Bronk and Phillips, 1932].

No naked-eye changes were observed in the soleus muscle when its sympathetic innervation was stimulated over long periods. Examined myographically the constant tension of a postural contraction was not seen to change during prolonged sympathetic stimulation (4 to 5 minutes). A passive stretch of 3 mm. in 0·25 seconds applied to the soleus tendon produced the same myotatic tension both in the resting muscle and throughout prolonged sympathetic stimulation (3 experiments). Under minimal stretch one could record with a string galvanometer the centrifugal firing of single anterior horn cells supplying the soleus muscle. Such preparations are at the best most unstable, but in at least three cases no modification was detected in the rate or rhythm of firing during sympathetic stimulation (Fig. 1).

The rate and rhythm of clonus and the number of units in synchronous activity was not influenced by sympathetic stimulation.

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Infra-red rays and ventilation. II. By Leonard Hill.

As already stated (*Proc. Physical Society*, Nov. 14, 1931), the nasal mucous membrane tends to become congested and the air-way obstructed as a reflex effect from stimulation of the skin by infra-red rays coming from a dull red or dark source of heat. The effect is pronounced only in those with a deflected septum or a chronic catarrhal and stuffy state of the nose. It can, however, be made evident in all normal people with widely open noses by the help of a screw nose-clip, adjusted to narrow the air-way and produce a significant degree of obstruction. Rays from incandescent sources acting on the same, or other parts of the body, antagonize the "nose-shutting" rays.

Any incandescent source can, however, be made a "nose-shutter" by means of a screen of acetyl cellulose (cellophane), gelatine or horny layer

of skin (desquamated), and also by a cloud of steam from the spout of a boiling kettle. Graphs prepared for me by Dr H. J. Taylor, by means of a rock-salt spectroscope and thermopile, show that all these screens absorb infra-red rays in the region about 30,000 A.U., and let through rays in the region 40,000–60,000 A.U. Cellophane, a "nose-shutting" screen, is transparent to ultra-violet and visible rays. White glass, on the other hand, opaque to the biologically active ultra-violet rays and transparent to visible rays, offers a large amount of protection from the "nose-shutting" rays; red glass affords less protection than white. The two kinds of glass absorb some of the rays only in the region of 30,000 A.U., red more than white; they also absorb infra-red rays longer than 45,000–50,000. It is clear then that ultra-violet and visible rays have nothing to do with either the "nose-shutting" or "opening" effects, and the "nose-closing" effect is caused by infra-red rays of small intensity other than those which cause "nose-opening." It is remarkable that, contrary to the action of a cloud of steam, or a layer of water which absorbs "nose-opening" rays, a film of water or an invisible screen of water vapour rising from a trough of cold or warm water makes the "nose-shutting" rays ineffective—hence the custom of putting troughs of water in front of stoves.

The sensitivity of the skin to infra-red rays shown by the nasal reflex is great; thus the nose may be shut on the side to which the septum is deflected when the author faces at a distance of 40 ft. a 300-watt tungsten filament lamp, screened by cellophane, and at a distance of 27 ft. when screened by a piece of the horny layer of the skin, 2 in. by 1 in., set in a sheet of cardboard. The sensitivity of the author is rivalled by that of others similarly incommoded by a deflected septum. Cooling the face by a fan or a cold surface at once antagonizes this "nose-shutting" effect.

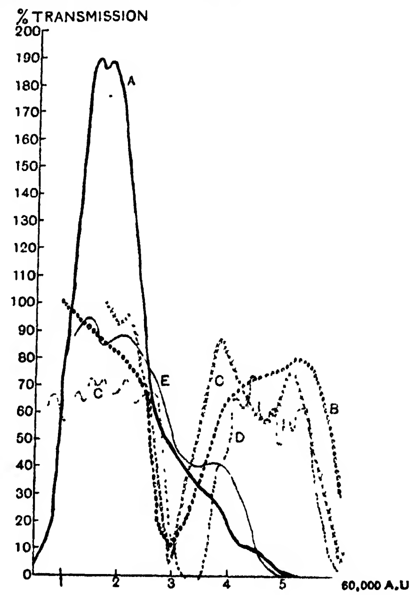
In the case of one subject the time of closing of the partially obstructed side of the nose was taken at various distances from the source, and the effect found to vary as $\sqrt{i}\,t$, where i = intensity and t = time.

On using different sized square holes in cardboard screens applied to the skin and keeping the distance of the source constant, the effect varied as $\sqrt{a}\,t$, where a = the area and t = time.

Rays from an incandescent source feel pleasanter and less scorching than those from dull red or dark sources of heat. A screen of cellophane, while taking away the feeling of heat, makes active the "nose-shutting" rays of any source, including the sun and an arc lamp. Increasing the energy output of a dull source in the short infra-red region increases

both the "nose-opening" effect and pleasanter quality of the rays. In the case of a bar electric fire an increase of current from 5 to 5½ amps. sufficed to convert this fire from a "nose-shutter" to a "nose-opener." The shortest infra-red rays, 8000–2800, penetrate the skin as deeply as red-rays do, and excite flushing and transudation, hence their less scorching effect. The "nose-shutting" infra-red rays cause stuffy feelings in susceptible people and probably contribute to catarrhal complaints. Ventilation with cool air counteracts their action, hence the need for the standard cooling power fixed by the author for the kata-thermometer.

A ——— Emission curve of lamp 300 watts.
B o o o Transmission of cellophane.
C × × × Water ·05 mm. in thickness.
D · · · · · Transmission of infra-red radiation by desquamated horny layer of skin
E ·——— White glass.



The inhibition of starch hydrolysis in the presence of glucose.

By Charles Reid.

Glucose is slowly formed later in the hydrolysis of starch by saliva [Evans, 1912], and its presence interferes to some extent with the action of blood diastase on starch [Narayana and Reid, 1930]. A filtered neutral solution of Merck's diastase was prepared of approximately the same diastatic activity as that of dog's blood and the inhibitory action of glucose on starch hydrolysis by this solution and by blood compared as follows:

A duplicate series of Erlemeyer flasks contained 1 c.c. of 0·1 p.c. soluble starch in 0·9 p.c. NaCl, 1 c.c. of 0·3 p.c. filtered Merck's diastase,

0·8 c.c. of 0·9 p.c. NaCl, and 0·2 c.c. of glucose of different p.c., the diastase being added last. One series was used for sugar estimation at once and the other after incubation at 38° C. for 30 minutes. For blood, a similar method was adopted using 0·2 c.c. blood with the chloride content of the solution in the flasks brought to the same figure as above which is near the optimum.

After incubation, the sugar content was determined by Maclean's method and the diastatic index determined according to the increased sugar content by the method suggested by Fyfe [1923]. The pH of the solution was 7.

Diastase (Merck)		Blood diastase	
Sugar p.c. in flask before incubation	Diastatic index	Sugar p.c. in flask before incubation	Diastatic index
0·0	49·2	0·007	51·0
0·01	48·4	0·015	49·6
0·02	42·8	0·026	35·4
0·035	38·4	0·039	34·8
0·044	35·2	0·047	33·2

Since the p.c. of starch in the flasks was 0·033, it is evident that substances related to the substrate produce inhibition when present in concentrations of the same order of magnitude as that of the substrate, and this is equally true for diastase (Merck) and blood diastase.

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Chromatolysis of motor-horn cells. By C. S. SHERRINGTON.

Certain degenerative changes (chromatolysis) in the microscopical structure of nerve cells are well known to ensue after amputation of the nerve fibre which springs from the cell. Motor-horn cells of the spinal cord of the monkey exhibit changes microscopically very similar to the above after transection of the cord headward from them. In this case, however, the manner of production of the change would seem different because neither is the axone fibre or indeed any part of the affected cell itself directly touched by the trauma. Thus, thirteen days after severance of the cord at 8th thoracic level in the monkey a number of the motor-horn cells in the hindlimb region, *e.g.* at 5th lumbar segment, are chromatolyzed. This is not due to any complication by myelitis. Clean rapid post-operative healing without a trace of sepsis has been the unbroken rule in the experiments.

McCouch [1924] has recorded finding chromatolysis in the spinal cells in a monkey accompanying a severe paraplegic state of 35 days' duration. In my own experiments a fortnight after the transection the chromatolysis is evident in a certain percentage of the ventral horn cells in the lumbo-sacral region, though not equally in all segments. In a number of the cells the perikaryon has lost its normal Nissl bodies to a large extent, a great deal of it has become hyaline, or even vacuolated in appearance; the nucleus has become displaced and eccentric, even so as to lie quite to one side.

A question arising is what, when the cell body is thus affected, is the condition of the motor nerve fibre which springs from it and, as we know, trophically and functionally depends on it. I have looked at the motor nerves of certain typical limb muscles [tibialis anticus, ext. long. digitorum, and gastrocnemius] and failed to find Wallerian degeneration in one case at the 17th day after the spinal transection. A functional test does, however, reveal at least as early as the thirteenth day distinct change in the motor nerve-muscle complex and in those very same flexor muscles. A single-shock stimulus applied directly to the motor nerve evolves a twitch contraction of subnormal tension value and sluggish to the degree of being more than twice the normal duration. The motor tetanus, evoked similarly by direct stimulation of the motor nerve, is correspondingly weak in tension, sluggish in relaxation, and its fusion rate is lowered, *e.g.* fairly perfect tetanic fusion at 22 stimuli per sec.

Functional defect in the motor nerve-muscle complex [Fulton and Sherrington 1932] accompanies therefore in these experiments in the monkey an abnormal state in certain of the spinal motor-horn cells which the microscope discovers in the corresponding region of the spinal cord. These dystrophic changes, of chromatolytic character, in spinal motor cells would seem, since consequent on a lesion distant from the cells themselves and many spinal segments further headward, to be examples of "transneuronal degeneration"; and the functional impairment of the nerve-muscle complex associated with it may be a further extension of, so to say, "transneuronal degeneration."

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Estimation of parathyroid hormone. By F. J. DYER.
(Pharmaceutical Society, London.)

The experiments described have been carried out with a sample of parathormone generously presented by Dr G. H. A. Clowes of Messrs Eli Lilly and Co. When parathormone is injected into rats, I find that it causes a rise in the urinary calcium which may last from one to four days. This is illustrated by the following figures for the urinary calcium of a group of twelve male rats fed on a uniform dry diet.

Day of experiment	Total volume of urine from group (c.c.)	Total Ca (mg.)
1st	90	7·6
2nd	72	16·9
3rd	58	20·2
4th	64	15·4
5th day each rat received 0·5 c.c. parathormone (s.c.)		
5th	68	46·9
6th	72	45·9
7th	56	29·9
8th	48	14·1

The average calcium excretion per day for the four days before the injection was 15·0 mg., and after the injection was 34·2 mg. The average rise per day was 19·2 mg. and reckoned per 100 g. rat was 0·96 mg.

The rise similarly determined in different groups of male rats for different doses of parathormone was:

Dose of parathormone per rat (c.c.)	No. of rats in group	Rise in urinary Ca, mg. per 100 g. per day
0·125	7	0·22
0·25	8	0·46
0·25	9	0·43
0·35	8	0·49
0·35	6	0·62
0·5	12	0·96
1·0	12	0·94

In two experiments the injection of 0·3 c.c. and of 0·5 c.c. parathormone produced no rise of urinary calcium.

The method of estimating parathyroid hormone proposed on the basis of these results is to take a group of twelve to twenty male rats, of weight about 140–180 g., which has been fed for some time on a normal diet. The group is divided into two parts, which are placed in separate metabolism cages, in which the rats are fed on a uniform dry diet, containing a known amount of Ca, *e.g.* 1 p.c. The urinary calcium is determined in the urine from each cage for a period of four to six days. The rats in the one cage are then injected with a dose of the preparation being tested, and the rats in the other cage are injected with the standard of reference with which the unknown is to be compared. The sample to

be tested must be matched against the standard, so that it produces about the same rise in urinary calcium as the standard. If in a first experiment the unknown sample does not do so, the figures given above are taken as a rough guide for readjusting the dose in a further experiment. There is some evidence that rats do not respond well to a second injection of parathormone. Calcium determinations were made by the method described by Collip and Clark (*J. Biol. Chem.* 1925, **64**, 485).

The behaviour of glycogen and lactic acid in normal and diabetic mammalian skeletal muscle under ischæmic conditions at body temperature. By CHARLES REID.

The animal (rabbit) was kept at rest for 1 hour before anæsthetization by intra-peritoneal amytal which lowers very slowly the glycogen p.c. in muscle [Evans, *et al.* 1931]. Repeated trials proved that glycogen and lactic acid contents of corresponding muscles carefully dissected out without twitching were nearly equal. To compare normal with ischæmic muscle, two or three muscles of one hindlimb were dissected out, cut on filter paper into two portions each—one for glycogen and the other for lactic acid estimation—and analysed. The animal was killed by incising the heart, placed in an incubator at 40° C. for 1 or 2 hours and the other hindlimb then used in the same way. In the case of dogs, an alternative method was also used: the tibialis anticus, for example, was removed, bleeding being controlled by an assistant, and cut rapidly into four pieces, two of which were used at once for glycogen and lactic acid and the others placed in stoppered bottles in an incubator at 40° C. for 1 or 2 hours before being subjected to analysis. This gave satisfactory results, as it was found that the glycogen and lactic acid distribution throughout the muscles used was practically uniform. Liquid oxygen technique was used in several experiments.

Methods.

Glycogen was estimated as glucose by the alcohol-KOH-alcohol method as used by Macleod, glucose by the Shaffer-Hartmann, and lactic acid by the Friedmann, Cotonio and Shaffer method.

Summary of results.

1. *Normal ischæmic muscle* (rabbit: dog). The glycogen content of normal muscles (rabbits) varied from 0·20 to 0·50 p.c.; at the end of 1 hour's ischæmia from 0·10 to 0·20 p.c. and after 2 hours' ischæmia from

0·07 to 0·16 p.c., while the lactic acid content increased from about 0·025 p.c. to nearly 0·10 p.c. in 1 hour and to 0·15 + p.c. in 2 hours' ischæmia at body temperature. Generally, the amount of lactic acid which accumulated in the above periods did not account for all the glycogen which disappeared from the muscle. Results from muscles of normal dogs were similar, but the initial glycogen content was often above 0·5 p.c. and lactic acid below 0·02 p.c.

2. *Diabetic ischæmic muscle* (dog). Several muscles were used from each depancreatized dog (ten) (blood sugar 0·17 to 0·37 p.c.). The glycogen content was lower than in normal well-fed dogs, but the lactic acid content was of the same order. During ischæmia the behaviour of glycogen and lactic acid was similar to that for normal muscle except for a slow increase in lactic acid in the case of muscles from diabetic dogs in very poor condition and with low muscle glycogen (0·1 to 0·15 mg. p.c.).

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Some properties of carbonic anhydrase, the CO_2 enzyme present in blood. By N. U. Meldrum and F. J. W. Roughton.

Blood corpuscles contain a catalyst which is exceedingly active in accelerating the formation of CO_2 from bicarbonate solutions by weak acids. The catalyst, in fact, hastens up both reactions of the reversible process $H_2CO_3 \rightleftharpoons H_2O + CO_2$. Till recently it had been rather supposed that the catalyst was hæmoglobin itself, but this we disproved two months ago by showing that the very pale filtrate obtained from ox-blood corpuscles after precipitation of the hæmoglobin by shaking with $CHCl_3$ or $CHCl_3$-alcohol mixtures had 50–80 p.c. of the activity of the original blood. For this new catalyst we have, at the suggestion of Eggleton, proposed the name "carbonic anhydrase," since, as shown below, it is found to possess the usual properties of enzymes.

More recently we have obtained the catalyst in solid form from the above filtrate, either by (*a*) alcohol-ether precipitation, or by (*b*) ultrafiltration followed by evaporation.

Preparation (*a*) is a nearly white powder, scarcely soluble in water but soluble in 0·02*M* $NaHCO_3$ on grinding. Preparation (*b*) is a reddish powder, easily soluble in water to a pale solution, which can be further purified by adsorption of the catalase present on $Al(OH)_3$, followed by ultrafiltration and evaporation.

Activity of the various preparations.

Preparation (*a*). 1 g. in 200 litres accelerated evolution of CO_2 from our standard bicarbonate-phosphate mixtures (0·2*M* $NaHCO_3$ + 0·2*M* phosphate buffer *p*H 6·8) five times.

Preparation (*b*). 1 g. in 80 litres gave sevenfold acceleration.

Hæmoglobin purified by Adair's method. 1 g. in 10 litres gave tenfold acceleration.

Stability to temperature.

Solution of preparation (*b*) in water and of lysed corpuscles were both stable to 30 minutes' heating at temperatures of 55° and below, but were both destroyed by 30 minutes at 65°.

Stability to pH *at room temperature* (20°).

All preparations have proved very stable in the range *p*H 6–10. Preparation (*b*) and lysed corpuscles were both stable for 30 minutes at *p*H 11 (borate buffer) and *p*H 12 (*N*/100 NaOH), but were destroyed at *p*H 13 (*N*/10 NaOH). They were stable for 30 minutes in citrate-phosphate buffer *p*H 4, but were destroyed at *p*H 3.

Chemical properties.

(i) Preparation (*a*) showed marked protein reactions (xanthoproteic, glyoxylic, Millon's and sulphur test), and also gave slightly positive α-naphthol test.

(ii) All the preparations of carbonic anhydrase free from hæmoglobin have so far been inhibited by 0·001*M* HCN, 0·001*M* H_2S and by CO at atmospheric pressure in the dark. The CO inhibition is light reversible. These results suggest that the enzyme is of the Warburg "hæmatin" type. With unpurified solutions of hæmoglobin, however, the results have been conflicting, and the whole matter is still open.

(iii) The enzyme is only partially (and variably) precipitated by saturation with $(NH_4)_2SO_4$. This suggests that the enzyme, if a protein, is probably a rather small molecule.

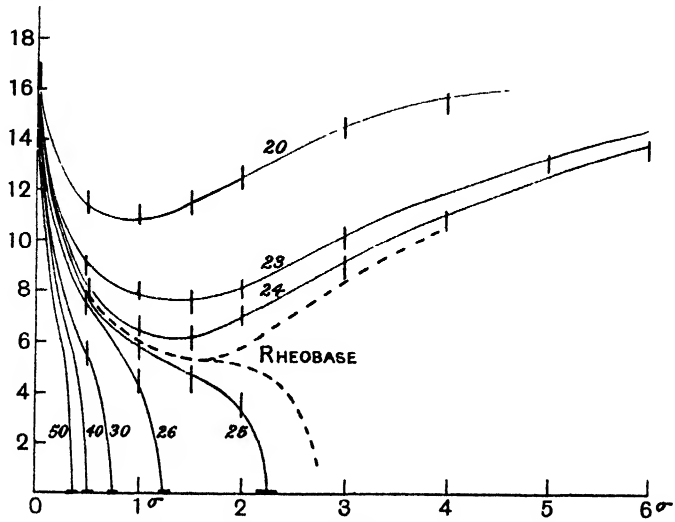
A new observation in the excitation of nerve and muscle.

By W. A. H. Rushton. (*Physiological Laboratory, Cambridge.*)

When a constant current passes through an irritable tissue a process develops which may culminate in excitation. This process can be investigated by superposing brief shocks at various instants and observing the threshold intensity, as has been done by Bishop [1928] and Erlanger and Blair [1931]. They worked with nerve and appear to have

restricted their observations to currents below the rheobase; in so far as brief constant currents are concerned, their results seem more or less consistent with a "condenser theory" of nerve excitation.

The present observations arose out of investigations upon the "α substance" of muscle, but since they apply equally to nerve they will here be described upon that more familiar tissue.



A constant current of given strength was passed through the nerve at various instants a very brief condenser discharge was superposed, and 0·2 σ afterwards the current was stopped. The figure shows the threshold strength of discharge (ordinates) at various instants (abscissæ) from the start of the current. Each curve corresponds to a different current strength as indicated. The curves are of two kinds dependent upon cutting the horizontal axis or not, *i.e.* whether the current is greater than the rheobase or not. The upper set are those hitherto investigated and confirm former work, the lower set show invariably (in passing from left to right) first the anticipated concavity upwards, then a point of inflexion and finally a "dive" towards the axis. In no case in nerve or muscle (α) has the curve been concave upwards where it meets the horizontal axis. The rheobase (broken line) is the limiting boundary of the two classes of curves. It is unique until it reaches 2σ or so, whereupon it branches as shown. Between the two branches is a region of instability in which no threshold point lies.

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Further observations on the function of the Thebesian vessels in the mammalian heart. By G. Stella.

In previous work [Stella, 1931] it was found that if the coronary arteries of a dog's heart be suddenly occluded, while the heart continues to perform work, the outflow of blood from the coronary sinus comes to a standstill after 10–12 beats. The inference was then made that the cardiac veins do not receive any blood directly from the ventricles, through the Thebesian vessels, though in free communication with them. The Thebesian vessels, therefore, during the systolic rise of intraventricular pressure must be obstructed at some point. Since only the coronary sinus was drained, it was still possible that some outflow occurred from the minor coronary veins. Moreover, it was impossible to decide whether and how far blood could enter the Thebesian vessels themselves. It was still possible, in fact, that there might be a to and fro movement perhaps reaching as far as the capillaries, and capable of carrying on a moderate blood supply to at least some part of the cardiac muscle, thus affording an explanation for those recorded cases in man, of survival after total occlusion of both coronary arteries.

In the present investigation the permeation of the Thebesian vessels by intraventricular blood was studied by colouring the latter with an easily visible dye, while direct penetration of the colour itself, *via* the aorta, into the coronary arteries was artificially prevented. In dogs, or cats, anæsthetized with urethane-morphine, or urethane, with the chest wide open, under artificial respiration, both coronary arteries were suddenly tied; the aorta was also tied beyond the origin of the brachiocephalic artery, and while the animal was being slowly bled through the latter, care being taken to keep the systolic intraventricular pressure fairly high, some c.c. of an 8 p.c. solution of Chicago blue were slowly injected into the left ventricle, or auricle. The blood coming out from the brachiocephalic was soon seen to be quite blue, but the surface of the ventricles and the superficial veins remained of their natural colour; the surface of the left auricle, however, particularly in the appendix, was soon seen to be blue. After 20–30 sec. the heart was brought to a standstill by means of a 10 p.c. KCl solution injected in the right auricle, and partly spread over the surface of the whole heart; after that, absolute alcohol was injected into the left ventricle, in order to precipitate all the dye which still remained in its cavity. At post-mortem there was no visible trace of colour in any part of the right ventricle. The left ventricle was, of course, very blue all over its endocardial surface, but cuts made in different parts

of its walls showed that the coloration did not penetrate more than 1 or 2 mm. Similar experiments were repeated, injecting the colour into the right ventricle, with practically the same results. Microscopic observations will subsequently be made of the route followed by the dye, in order to decide to what the small penetration of colour referred to above is due.

For the present it seems justifiable to conclude that if penetration does occur under these experimental conditions into the Thebesian vessels, it is a very limited one: it does not reach the veins, and leaves the great mass of the heart muscle completely unsupplied.

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The co-ferment activity of adenylpyrophosphate in lactic acid fermentation. By T. H. Milroy.

Recent work by Meyerhof and Lohmann [1931 *a*, *b*] has directed attention to the part played by adenylpyrophosphate (or adenosinetriphosphate) as the organic component of the co-ferment of lactic acid fermentation. In this brief preliminary communication attention will be directed to the action of this body at low temperatures and also to the part which it plays in ferment extracts from muscle in a state of action rigor (monoiodoacetate poisoning) or from muscle undergoing post-mortem changes. Ferment extracts were inactivated either by repeated extraction of rabbit's muscle and preserving the extracts for some time at low temperatures, or by dialysis, or by a combination of both methods. When adenylpyrophosphate or certain of its derivatives is added to such inactive extracts, reactivation, as shown by esterification and lactic acid production, occurs.

Experiments were carried out at a low temperature (about 8°) because in this way the early changes in reactivation can be detected. The actions of adenosinetriphosphate (ap.) and its deaminated derivative, inosinetriphosphate (ip.) were both investigated.

Action at 8°. A dialysed muscle ferment extract was added to a solution containing starch, orthophosphate and one or other of the two nucleotides, both solutions being at 8°. Within one minute the action was stopped by addition of trichloroacetic acid. The phosphorus distribution in the solutions before and after admixture was determined. Three analyses were made in each case, namely, the orthophosphate, the in-

crement in orthophosphate on 10′ acid hydrolysis, and the total phosphorus. The results were as follows (mg. P per c.c.):

	Before mixing (same for ap. and ip. additions)	After mixing (one minute's action) With ap.	With ip.
"Ortho"	0·47	0·47	0·46
10′ acid hydrolysis	0·53	0·47	0·46
Total	0·56	0·56	0·55

The same amount of phosphorus (0·03 mg. per c.c.) was added in the form of ap. and ip. In this short interval at 8° there is no encroachment on the "ortho" value, but in both cases the "pyro" portion originally present has passed into more stable form (hexose ester). Analyses at later periods show the progress of esterification and lactic acid production. Stated in terms of millimolar concentrations of P in ester form and lactic acid at later periods of incubation (8°).

	With ap. 25′	60′	17 hr.	With ip. 25′	60′	17 hr.
Ester	*2·3*	*4·1*	*2·0*	*1·6*	*2·9*	*2·1*
Lactic acid	1·4	2·7	9·2	1·0	1·7	6·5

At higher temperatures (18°, 25°, etc.) the same changes are to be observed but occurring within shorter periods. The earliest change, disappearance of the "pyro" fraction, has only been observed in cooled mixtures.

The action of ferment extracts from muscle undergoing post-mortem changes and from muscles in a state of action rigor (monoiodoacetate) has also been studied. Inactive extracts of the former may be readily re-activated both as regards esterification and glycolysis by the addition of the pyro-nucleotides. Dialysed inactive extracts of monoiodoacetate muscle can readily be re-activated, so far as esterification is concerned, by addition of adenosine triphosphate, but only very slight, if any, lactic acid production is brought about. If the monoiodoacetate muscle extracts have not been completely inactivated by dialysis or otherwise, the addition of adenosine-triphosphate will not only increase esterification but will also bring about a slight increase in lactic acid production. Such extracts never recover the glycolytic power of normal muscle extracts whether the co-ferment addition is boiled extract of normal muscle or any of the nucleotides or their derivatives. The disturbance in the ferment system in this form of poisoning is certainly not solely in the co-ferment, but also in the colloidal non-dialysable component.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

June 11, 1932.

Distribution of lactate between blood corpuscles and plasma.

By S. C. Devadatta. (*Physiology Dept., Univ. of Edinburgh.*)

Both oxygenated and reduced blood were subjected to different pressures of carbon dioxide: as the pressure of carbon dioxide was increased the ratio of concentration of lactate in corpuscles to that in plasma increased. For the same pressure of carbon dioxide the lactate ratio c/p was higher in reduced blood (0·80) than in oxygenated blood (0·45). In other words the concentration of lactate in the corpuscles of venous blood (9 mg. per 100 g.) is greater than in those of arterial blood (6·8 mg. per 100 g.), the concentration of lactate in whole blood being 10·8 mg. per 100 g. Therefore in each respiratory cycle there is a to and fro lactate shift—about 8 p.c. of the amount of lactate present in blood takes part in this to and fro shift.

Concentration of lactate in whole blood. When the concentration of lactate in whole blood is low, the concentration of lactate in the corpuscles is about 75 p.c. of that in plasma. There is a progressive drop in the lactate ratio c/p as the concentration of lactate in whole blood increases after exercise. In fatigued subjects the lactate ratio c/p drops to 0·50.

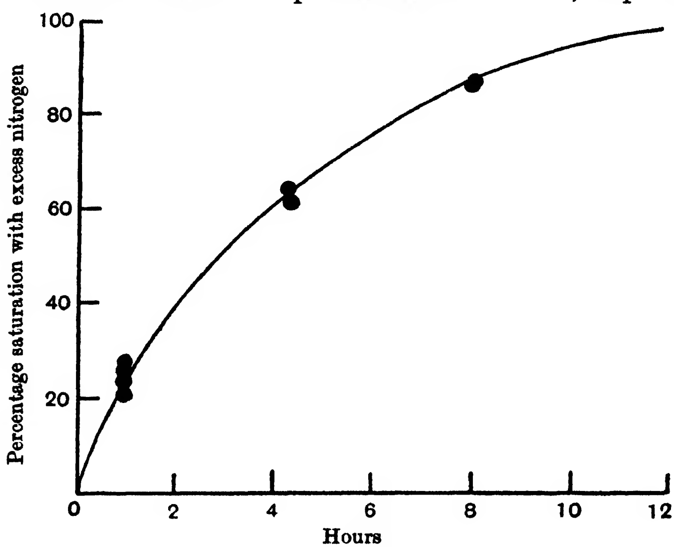
Neutral sodium lactate solution was added to blood maintaining the osmotic pressure, the *p*H and the percentage of the corpuscles constant; the same effect was observed. As the concentration of lactate in plasma increases, the increase is in the corpuscles as well but not to the same extent. The lactate ratio c/p drops to 0·30.

If lactic acid was used instead of sodium lactate in experiments of this type, the change in the c/p ratio was slightly less for equal increases in lactate content. This is attributed to neutralization of the acid by hæmoglobin.

The lactate ratio c/p 0·50 in fatigued subjects is due to the combined effects of all three factors above mentioned. If blood treated with lactic acid is equilibrated with a high pressure of carbon dioxide (150 mm. Hg), the lactate ratio c/p rises from 0·36 to 0·50, which is that of fatigued subjects.

Rate of saturation of goat's tissues with excess gaseous nitrogen during exposure in vivo to increased atmospheric pressure. By J. Argyll Campbell and Leonard Hill. (*Preliminary Communication.*)

We have investigated the rate of saturation *in vivo* of goat's brain, liver and bone marrow with excess gaseous nitrogen under + 3, + 4 and + 5 atmospheres pressure. Some results for fatty bone marrow (about 90 p.c. fat) during exposure to + 3 atmospheres pressure of air are shown in the chart. The marrow is about 25 p.c. saturated in 1 hour, 60 p.c. saturated



in 4 hours and 90 p.c. saturated in 8 hours. This slow rate of saturation is due to the low blood supply to fatty marrow and the slow rate of diffusion of nitrogen through this tissue.

The nitrogen in the marrow is estimated with the method previously described [Campbell and Hill, 1931]. The goat is killed and the marrow placed in the special apparatus and thus shut off from the external air before decompression. The apparatus gives accurate results, when tested against gas pumps, for nitrogen in solution in various fluids, *e.g.* water, olive oil, liquid paraffin, acetic acid, formic acid.

The nitrogen content of the marrow under normal atmospheric pressure is estimated from the fat content and the fact that pure marrow fat contains about 5 c.c. of nitrogen per 100 c.c. of substance and the remaining tissue about 1 c.c. per 100 c.c. under normal conditions.

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The effects of adrenaline and ephedrine upon certain tissue gas tensions. By J. Argyll Campbell. (*National Institute for Medical Research.*)

The gas tensions under the skin and in the abdominal cavity were estimated as previously described [Campbell, 1924]. The rabbits (about 2 kg.), which were not anæsthetized, were tested with a subcutaneous injection of ephedrine sulphate and a week later with adrenaline chloride. The tensions recorded in Tables I and II were obtained about 4½ hours after the injection of the drugs. Omitting the figures for Rabbit No. I,

Table I. Effect of hypodermic injection of ephedrine sulphate upon tissue gas tensions.

		CO_2 tensions mm. Hg				O_2 tensions mm. Hg			
		Skin		Abd. cav.		Skin		Abd. cav.	
Rabbit no.	Dose mg.	Before inj.	After inj.	Before inj.	After inj.	Before inj.	After inj.	Before inj.	After inj.
I	2	58	49	54	49	23	28	34	34
II	4	49	49	48	47	21	26	39	41
III	17	52	49	50	49	23	29	34	38
IV	53	56	51	52	47	19	23	38	40
Average		54	49	51	48	21	26	36	38

Table II. Effect of hypodermic injection of adrenaline chloride on tissue gas tensions.

		CO_2 tensions mm. Hg				O_2 tensions mm. Hg			
		Skin		Abd. cav.		Skin		Abd. cav.	
Rabbit no.	Dose mg.	Before inj.	After inj.	Before inj.	After inj.	Before inj.	After inj.	Before inj.	After inj.
I (died)	2	50	118	50	140	22	1	35	10
II	1	52	59	51	53	21	12	37	34
III	2	49	65	50	58	20	13	30	24
IV	2	57	85	53	62	15	4	38	28
Average (living animals)		52	69	51	58	19	10	35	29

which died under the effects of adrenaline chloride, it will be noted that whilst this substance produced an average fall of oxygen tension of 9 mm. Hg under the skin and of 6 mm. Hg in the abdominal cavity, ephedrine produced an average increase in oxygen tension of 5 mm. Hg under the skin and of 2 mm. in the abdominal cavity. Further adrenaline increased the carbon dioxide tension by 17 mm. Hg under the skin and by 7 mm. Hg in the abdominal cavity, whereas ephedrine decreased this tension by 5 mm. Hg under the skin and by 3 mm. Hg in the abdominal cavity. The results with adrenaline are characteristic of constriction of blood vessels and those of ephedrine are characteristic of dilatation. The

conclusion is that the distribution of blood is different under the influence of the two drugs. Chen and Schmidt [1924] found that the cutaneous vessels were dilated by ephedrine; at the same time those of the kidney and intestine were constricted.

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The excretion of urea by the elasmobranch and mammalian kidney. By J. D. S. Cameron.

Xanthhydrol as a precipitant for urea was first suggested by Fosse. It has since been used by Policard, Chevalier and Chabanier, Stübel, Oliver, Piras, Walter, and Hollman to demonstrate the excretion of urea. The results obtained by these workers were inconclusive and were applicable to a comparatively limited range of animals.

In the series of experiments reported, a comparative study of the excretion of urea has been attempted. Use has been made of the kidneys of elasmobranch fishes (skate and dogfish), frogs, rats, mice, guinea-pigs, rabbits, cats, dogs, monkeys and man.

The results obtained in the elasmobranchs varied with the demands placed upon the kidneys. In the kidneys of fish killed immediately on removal from sea water, dixanthylurea crystals were found in the glomerular capsules, lumina of tubules, blood vessels and interstitial tissue. In those injected with urea the tubular epithelium also showed crystals.

In the kidneys of all the mammals employed abundant crystals were seen in the glomerular capsules, tubule lumina, blood vessels and interstitial tissue, but in no kidney were crystals found in the tubular epithelial cells. This applied to injected and uninjected animals and to pathological conditions in man in which the blood urea concentration was raised to levels varying from 105–320 mg. per 100 c.c.

In elasmobranch physiology urea plays a double rôle—acting as a medium of nitrogen excretion and as the regulator of blood osmotic tension. In the mammal it serves the first function only. The excretion of urea is consequently of greater urgency in the elasmobranch than in the mammal, and to this fact is attributed the difference existing between the mode of action of the kidney as regards urea in the elasmobranch and in the mammal.

The tolerance of medulliadrenalectomized and adrenalectomized rabbits to intravenous injections of glucose. By CHARLES REID.

The usual dosage and routine adopted were practically similar to those used by Orr-Ewing [1931], *i.e.* 1·0 g. glucose per 100 c.c. blood volume as calculated by the formula of Dreyer and Ray [1911] was injected into the ear vein from an accurate 5 c.c. syringe.

$$\text{Blood volume in c.c.} = \frac{\text{Weight in g. } 0{\cdot}72}{2{\cdot}37}.$$

(1) *Medulliadrenalectomized rabbits.* This series comprised 15 rabbits. Their glucose tolerance was determined by repeated tests in each rabbit (fasting) before operation and after the second operation for destruction of the medulla by scraping or cauterization. On the whole, the slightly more rapid fall towards the pre-injection level of the blood sugar in the course of 1 to 1½ hours in the medulliadrenalectomized rabbits suggested that in the absence of or with the decreased amount of adrenaline in the body the sugar tolerance of the animals was slightly raised. This series of observations, however, requires further study.

(2) *Adrenalectomized rabbits.* This series also comprised 15 rabbits. Glucose tolerance tests were done as for (1) with the usual interval of from 2 to 4 weeks between the aseptic adrenalectomics on each rabbit. Glucose tolerance was increased after the second adrenalectomy, the blood sugar having increased about 60 to 90 mg. p.c. 15 minutes after the injection as compared with an increase of from 100 to 140 mg. p.c. for the rabbits before operation. Three rabbits of the series gave indefinite results, all females, of which two had become pregnant before being segregated for the series.

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Nerves of the epidermis. By M. H. KIDSTON and D. WATERSTON.

Thin sections of epidermis can be cut from the fingers or other parts of the surface without any pain, the level of the section extending down to the stratum mucosum.

Examination of portions thus removed has shown that nerves have been divided and their terminals can be seen among the epithelial cells, similar to those which have been described from sections of the entire skin.

It is concluded that the nerves of the epidermis are nerves of the specific sense of touch and do not convey impulses which cause pain, and therefore are in the same category as other nerves of special sensation.

The activity of the optic ganglion of Dytiscus marginalis.

By E. D. Adrian.

Hartline and Graham [1932] have recently published records of the discharge of impulses in the optic nerve fibres of *Limulus*. These fibres lead directly from the photoreceptors and their activity is not complicated by synaptic effects in the retina. The impulse discharges in each fibre agree very closely with those in the sensory fibres from other kinds of receptors, *e.g.* the stretch receptors in frog's muscle.

It is interesting to compare these results with records from the optic nerve of the water beetle (*Dytiscus marginalis*), for this nerve leads from the optic ganglion at the back of the eye and the discharge in it bears evidence of interaction, as in the vertebrate optic nerve. This is shown by the appearance of synchronous or grouped discharges in which the impulses in different fibres occur in definite groups instead of independently

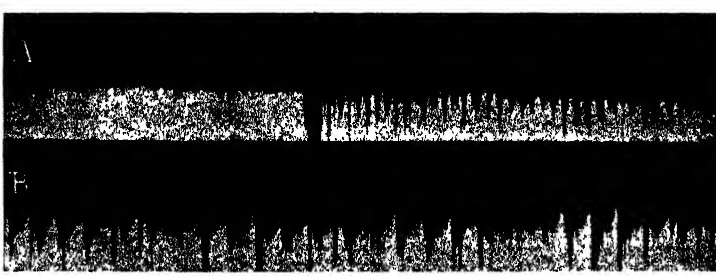

Fig. 1. Impulses in optic nerve of *Dytiscus* showing the tendency to grouping: A, light turned on suddenly; B, another preparation, light on for three seconds. The visual field was not uniformly illuminated and the grouping is incomplete. Condenser coupled amplifier with 0·005 mf. coupling condenser to exclude slow changes. Time marker (white lines) gives 0·25 sec. intervals.

(Fig. 1 A and B). The conditions which favour this type of activity are (*a*) even illumination of the visual field, and (*b*) a bright light or a sensitive (dark adapted) eye, but neither is essential.

The groups of impulses are usually discharged at a rate of 15–18 a sec. and each is accompanied by a slow potential change. When a direct-coupled amplifier is used the slower changes appear as a smooth curve on which the impulses are superimposed, the discharge occurring as the ganglion electrode develops a negative potential (Fig. 2 A). Slow waves

at 5–6 a sec. may appear when the eye is in darkness (Fig. 2 B), but there is then only an occasional impulse in the nerve, as though there were

Fig. 2. Slow potential changes: A, during even illumination; B, in darkness. In A the impulses appear as the optic ganglion becomes negative. Direct coupled amplifier, upward movement of shadow when distal electrode (on ganglion) is negative. Time marker gives 0·25 sec.

a change of potential in the ganglion too slow to excite the nerve fibres. Fig. 3 shows the effect of increasing illumination.

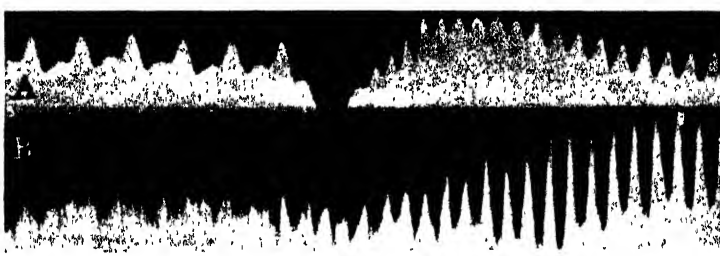

Fig. 3. Effect on slow changes of increasing light: A, sudden illumination; B, another pre paration, gradual increase of light causing development of waves. Condenser coupled amplifier (1 mf. condenser). Time marker (0·25 sec.) same in both records.

This kind of activity resembles that first shown by Fröhlich [1913] in the cephalopod optic nerve and later by Adrian and Matthews [1928] in the eel. The slow changes recall the slow respiratory waves in the abdominal ganglia of *Dytiscus* [Adrian, 1931], but here the structures are so small that it is hard to say where the effect is developed.

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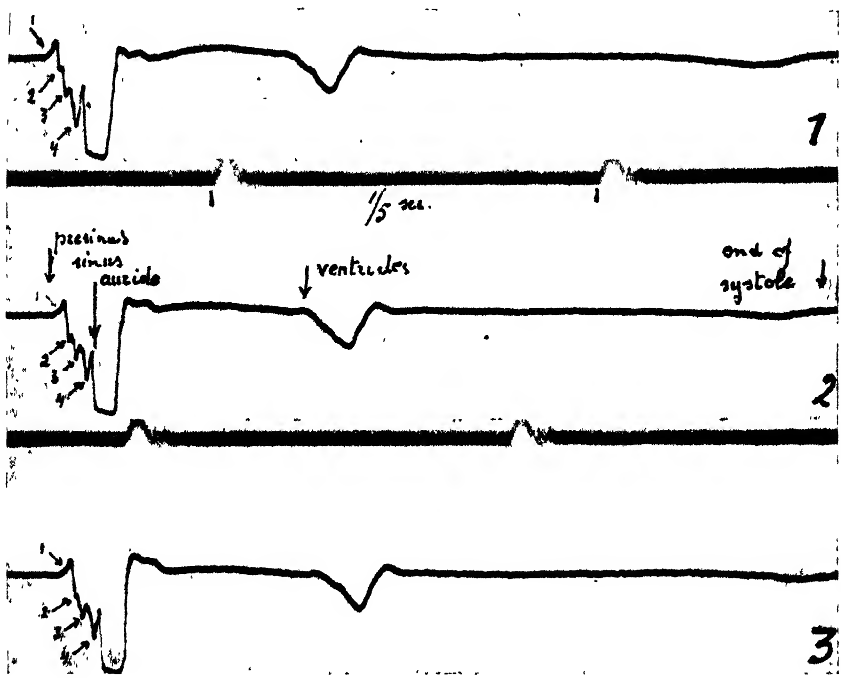
The pacemaker of the mammalian heart. By P. Rijlant. (*Brussels.*)

The action current of the pacemaker of the mammalian heart has been recorded by the string galvanometer [Rijlant, 1925; Athanasiu, 1926], but the records did not allow any further study of this activity.

Latterly the Erlanger and Gasser cathode ray oscillograph has been used, and evidence obtained as to the complex and oscillatory activity of the pacemaker both in the heart *in situ* and in the isolated auricle.



Dog.—Chloralosane. Heart exposed. Electrodes in the presinus region. Three successive normal beats: 1, presinus wave, 2, 3 and 4, sinus waves. The series of waves have the same shape and duration in successive beats.

A series of electric changes were observed, the first wave being somewhat individualized. This initial activity was called "presinusal," and the following ones "sinusal" [1928].

Recent observations of Bradley Patten on the development of the bird embryo indicate that in certain stages of the development the heart beat starts in the duct of Cuvier, and then is propagated to the sinus, auricles and ventricles. The activity of the sinus venosus appears in later stages of the development. These observations of Bradley Patten can

be compared with the findings in the mammalian heart. The presinus where the beat starts is the embryological equivalent of the duct of Cuvier, and the Keith Flack node is the remnant of the sinus.

The activity of the pacemaker of the mammalian heart has been explored further by using a method of continuous cathode ray oscillography; by this means successive cycles of the heart activity may be recorded and their characteristics compared.

In the intact heart, *in situ*, of the dog, cat or rabbit, two electrodes are thrust into the sinus and presinus. The action currents are amplified 500,000 to 5,000,000 times and lead to a cathode ray oscillograph. Continuous records are made at a speed averaging 50 to 125 cm. a second.

In these records a pre-auricular action current is observed; its beginning precedes the auricular action current by about 5 to 20 σ. This interval can be increased to 200 σ by cooling the heart; partial or total sino-auricular block may be observed in these circumstances.

These results, from the study of successive cardiac cycles, corroborate our earlier experiments. The series of waves corresponding to the activity of the "presinus" and "sinus" have the same shape in successive beats, and their different component waves maintain the same height and direction.

Under normal circumstances, the action current of the pacemaker (presinus and sinus) is composed of a series of electrical changes of constant shape and duration.

The activity of the pacemaker of the mammalian heart is due to the summation of a series of activities localized in the different parts of the pacemaker.

Calcium chloride aciduria. By P. W. CLARKSON. (*Physiology Dept., Univ. of Edinburgh.*)

The aciduria induced in a human subject by oral administration of calcium chloride was studied quantitatively by measurements, in successive urine samples, of (*a*) *p*H (hydrogen electrode), (*b*) the inorganic phosphate content, (*c*) the titration value with NaOH and HCl to phenolphthalein and methyl red respectively. The normal *p*H of the subject's urine during the fasting condition was 6·1. 1 g. of $CaCl_2$ $6H_2O$ administered in solution produced a change to about 5·8 in 2 hours, the effect then passing off in about the same time. A dose twice as large produced a change to *p*H 5·1 in 6 hours, falling thereafter. A dose of 20 g. produced

its maximum effect in the same time, but the intensity was greater, a *p*H of 4·5 being reached.

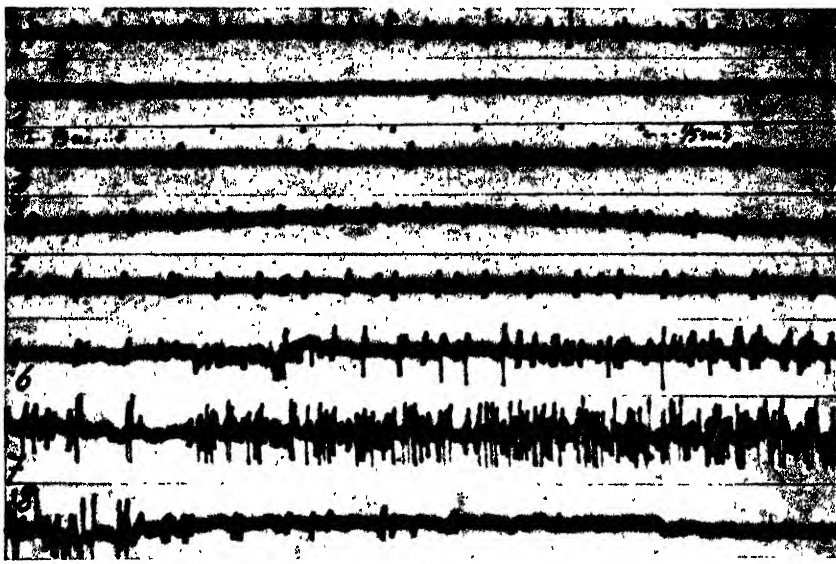
The buffer value of the urine (titration range between methyl red and phenolphthalein) was usually accounted for in large measure by the inorganic phosphate content (confirming Leathes). Administration of creatine by mouth in doses of 10 g. has been found temporarily to inhibit the excretion of phosphate, the effect passing off in 3–4 hours. This confirms the experience of Brown and Imrie dealing with anæsthetized cats. A dose of 1 g. of $CaCl_2$, followed 90 minutes later by 10 g. of creatine, produces an aciduria comparable in extent and duration with that resulting from 20 g. of calcium chloride alone. This enhancement is attributed to the lessened buffering power of the urine deprived of phosphate.

The effect of thyroid and anterior pituitary on the thyroid and testis of the rat. By Y. Sidki. (*Physiology Dept., Edinburgh.*)

Extracts of thyroid and anterior pituitary lobe were administered both subcutaneously and by the mouth. The amounts given were thoroughly mixed into a well-balanced diet rich in vitamines. The amounts given to each animal were 0·05 g. of dried thyroid and 0·1 g. of dried anterior pituitary in the form of extracts. Subcutaneous injections were given to other animals every other day. The effect of thyroid feeding on the testis was to produce degeneration of the seminiferous tubules, the epithelium almost completely disappearing. The interstitial cells showed hypertrophy. The effect of anterior pituitary feeding was to produce slightly increased spermatogenesis and indications of degenerative changes in the interstitial tissue. Administered hypodermically a similar effect is produced on the testis, but the effect is less evident than with feeding. This may possibly be due to the smaller amount of extract administered. The effect of thyroid administration on the thyroid gland was to cause the appearance of inactivity of the vesicles, which are greatly distended with colloid, their epithelium being much flattened and sometimes hardly visible. Feeding with anterior pituitary, on the other hand, causes the appearance of increased activity. The vesicles are small and contain little colloid and their cells are cubical or columnar. Administration by injection exhibits similar effects, but they are less marked. The results are illustrated by microscopic sections stained by Heidenhain's hæmatoxylin, and some counterstained with chromotrope. The thyroid is stained with anilin blue and orange *G*.

Muscular tonus in man. By P. Rijlant. (*Brussels.*)

The action currents of human muscles during voluntary contraction have been recorded by Adrian, but no electric changes were observed in the quiescent muscle. Buytendijk, Liddel and Fulton, Dusser de Barenne, Wachholder, Rademaker and Hoogerwerf, and Adrian, recorded the action currents of the muscles of the cat during decerebrate rigidity.



Man.—Extensor digitorum communis. Tracing 1, normal tonus waves. Tracing 2, the muscle is shortened: no tonus waves. Tracings 3, 4 and 5, progressive return to normal length of the muscle (normal tonus waves in 5). Tracings 6 and 7, progressive lengthening of the muscle. Tracing 8, progressive shortening of the muscle.

The electric activity of muscle can be recorded by the cathode ray oscillograph. Two steel needles are thrust into the muscle, through the skin, and connected to the input of an amplifier. The action currents, amplified 500,000 times, are led to a cathode ray oscillograph and the movements of the spot are recorded on film or bromide paper moved at uniform speed. The experiments were made on man and on cats.

Electric changes can be recorded from all relaxed muscles either extensor, flexor, adductor or abductor; if very few fibres are active the impulses form single series of uniform amplitude and frequency. The average frequencies observed range between 10 and 20 a second in the

extensor digitorum communis, biceps and quadriceps of man. In the relaxed muscle very few muscle fibres are active simultaneously.

During progressive lengthening of an extensor or flexor muscle fresh series of impulses appear, due to the activity of new groups of fibres, and the frequency of the initial series of discharges increases up to a maximum of from 50 to 60 a second.

Simultaneous lengthening of two antagonistic muscles increases simultaneously their electric activity. The normal activity is restored when the muscle is relaxed.

Shortening of a muscle diminishes the frequency of the single series of impulses, diminishes the number of active fibres and diminishes the height of single impulses. During considerable shortening no appreciable activity can be recorded.

Adrian has recorded regular series of impulses in human triceps during voluntary contraction. These electric changes can be compared with those due to the activity of the resting muscle. During voluntary contraction series of impulses of increasing frequency appear but do not interfere with the initial activity of the muscle. These new series of impulses have the same height and initial frequency as the series of impulses of the quiescent muscle but originate in other fibres; their frequency is rapidly increased to from 50 to 80 a minute. During voluntary contraction no inhibition of the electric changes which occur in the resting muscle can be observed, but when the contraction is released these changes disappear during 1 to 2 seconds and then are progressively restored.

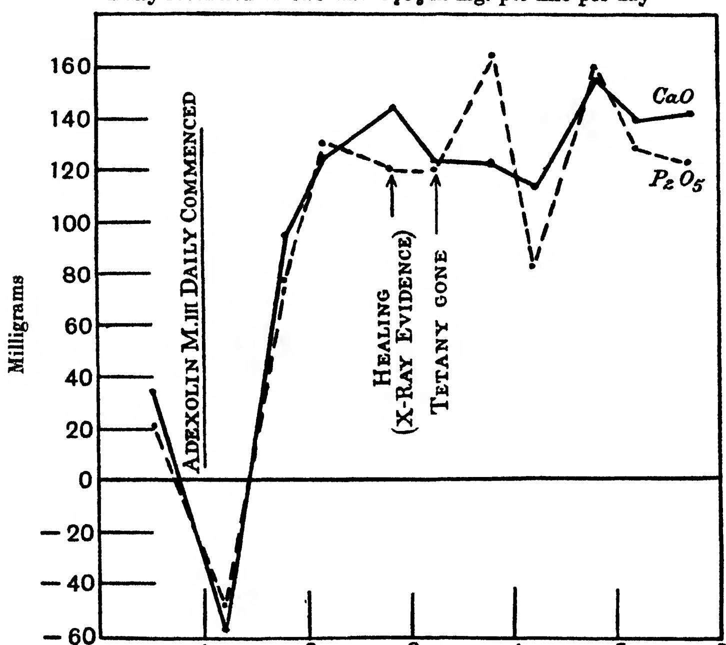
During voluntary contraction of a muscle the electric changes disappear in the antagonist muscle; when the contracting muscle is relaxed the inhibited muscle recovers its activity which is considerably increased during 1 to 2 seconds.

These experiments are wholly confirmative of the findings of Sherrington and Adrian on the decerebrate cat. They show that in every resting muscle in man electric changes can be recorded, and that this resting activity can be increased or diminished by lengthening or shortening of the muscle. This activity is not inhibited during voluntary contraction of a muscle but is inhibited during 1 to 2 seconds when the muscle is released; it is inhibited in the antagonistic muscle.

Difference between the retentions of calcium and phosphorus as a factor in the production of infantile tetany. By F. J. Ford, S. G. Graham and Noah Morris. (*Biochemical Laboratory, Department of Medical Pædiatrics, Univ. of Glasgow.*) (*Preliminary communication.*)

Recent experimental work by Hess and others [1931] seems to indicate that an important factor in the onset of tetany in rachitic animals is an increase in the retention of phosphorus without corresponding increase in that of calcium. Rominger and his co-workers [1931] assert that infantile tetany, so commonly associated with rickets in the young child, is also due to relative increase of phosphorus over calcium retention. In two children, E. B. and J. W., both the subjects of

Daily retention of CaO and P_2O_5 in mg. per kilo per day



Time in weeks

Fig. 1.

active rickets, and E. B. with latent tetany, calcium and phosphorus retentions were determined before and after the administration of vitamin D (adexolin). The retentions of calcium and phosphorus, which in both instances were increased, ran parallel to one another, although in E. B. signs of latent tetany did not disappear till 16 days after vitamin

D was commenced. Fig. 1 shows the result in E. B. Phosphate was administered to two children: W. B. who had marked osteoporosis, was given NaH_2PO_4 and J. W. with healing rickets received Na_2HPO_4 over a period of 3 weeks. In both cases the retention of phosphorus was markedly increased, while the retention of calcium remained subnormal, but there was no evidence of tetany latent or active.

TABLE I. Effect of phosphate ingestion on calcium and phosphorus retention.

	Retention in mg. per kg. per day	
	CaO	P_2O_5
Case I (W. B.)		
Before giving phosphate	− 93	− 9
NaH_2PO_4 g. 2·5 daily	+ 4	+178
Case II (J. W.)		
Before giving phosphate	+123	+166
Na_2HPO_4 g. 3·0 daily	− 16	+207

From these results it would seem that in the pathogenesis of infantile tetany associated with rickets, excess retention of phosphorus is not a prime factor.

Two of us (N. M. and S. G.) are in receipt of personal grants from the Medical Research Council to whom we express our thanks for defraying the expenses of this research.

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Blood cholesterol and red blood cell fragility following bilateral adrenalectomy. By CHARLES REID.

Numerous investigators have attempted with indifferent success to connect the adrenal glands with lipoid metabolism by blood cholesterol estimations before and after adrenalectomy, but the influence of the resulting hæmoconcentration which may follow bilateral adrenalectomy in many cases has not been taken into account in their assessment of the results.

Accordingly, blood cholesterol (Leiboff's method as described by Hawk [1931]) was determined in 20 rabbits by repeated observations in duplicate extending in each over a period of several weeks. Their adrenal glands were removed with intervals of 3 to 4 weeks between the operations, the blood cholesterol being determined several times between the operations and after the second operation at intervals of several days, and

the hæmoconcentration controlled at the same time by determinations of specific gravity, total solids, p.c. hæmoglobin and enumeration of red blood cells per cm. No significant variation in the blood cholesterol content, not observed before the operations, occurred after the first and second operations except such as could be accounted for by the increased concentration of the blood observed in a number of the rabbits after the second operation.

Red blood cell fragility appeared to be slightly decreased in a number of cases after the removal of the second adrenal, hæmolysis occurring in 0·5 p.c. saline before operation and in 0·45 p.c. saline after operation.

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Hawk, P. B. and Bergeim, O. (1931). *Practical Physiological Chemistry*, p. 441. J. and A. Churchill, London.

The sensitization of the guinea-pig's uterus to pituitrin. By R. E. Illingworth, P. G. Marshall and J. M. Robson. (*Macaulay Laboratory, Institute of Animal Genetics, Edinburgh.*)

The reactivity of the isolated guinea-pig's uterus to pituitrin is increased by the addition to the bath of

(1) α- and protein-free preparations from late and early pregnancy urine containing gonadotropic substances and made by alcohol precipitation;

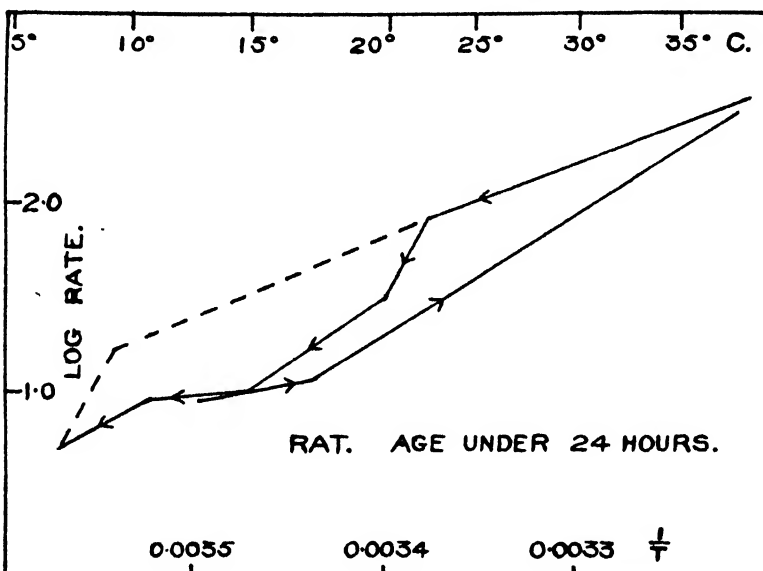
(2) Preparations as in (1) boiled for several minutes (which destroys gonadotropic hormones);

(3) Similar preparations made from the urine of non-pregnant women and containing no detectable amounts of gonadotropic substances.

Sensitization has been obtained by the addition to the bath (100 c.c.) of 0·25 mg. of substance (obtained from pregnancy urine) containing 50 luteinizing units. In other experiments even larger doses of gonadotropic substance were without effect. The sensitization is not due to changes in *p*H and persists after change of solution. It is concluded that the sensitization is not due to gonadotropic hormones, and that the factor responsible for it is not specifically excreted during pregnancy.

Experiments on the temperature coefficient of heart activity.
By H. TAYLOR and B. P. WIESNER. (*Macaulay Laboratory, Institute of Animal Genetics, Univ. of Edinburgh.*)

It has been shown that the young rat for the first few days of its life is poikilothermic; this makes it a suitable material for the study of the temperature coefficient of the heart. It can be kept for a considerable time at 0° and on warming up shows no ill effects. The heart rate has been recorded by means of an amplifier, the electrodes being two very thin copper wires placed in or near the heart. The temperature of the animal has been recorded by a copper-constantine couple, one junction



in the rectum and one in the thorax. The animal was anæsthetized by 0·5 milligram of dial and immersed in water at various temperatures.

The graph shows the relationship between the log of the rate and the reciprocal of the absolute temperature. On cooling down from 38° a straight line is obtained up to approximately 23° when there is a sharp break, after which the curve again goes in a straight line from 21° to 15°. The lower values cannot be fitted on to a line and are apt to be irregular. The ventricle appears to stop completely about 5°. On rewarming the heart begins to beat again at approximately 7°, but below 12° the rate is difficult to estimate owing to arrhythmia. On the heating-up curve

there is discontinuity at 18°, but afterwards the graph runs straight to 38° at which place the cooling curve is cut.

In some animals the cooling curve does not show discontinuity at 23° but gives a straight line to 10° as shown by the dotted line in the diagram.

The stoppage of the heart at low temperatures and the return of the beat on warming can also be demonstrated by transillumination.

Some factors affecting the development of the testis. By J. M. Robson and H. Taylor. (*Macaulay Laboratory, Institute of Animal Genetics, Univ. of Edinburgh.*)

Reports of experiments with the gonadotropic hormones from pregnancy urine on testicular size generally agree that these hormones accelerate the growth of the testicle. Pituitary extracts are also supposed to have the same effect.

In the experiments reported here the effects of implantation of anterior pituitary lobe substance into rats have been noted. The animals were 3 or 4 weeks old at the beginning of the experiments and were implanted on alternate days with 50 mg. of bovine pituitary, except for the first three implantations when 25 mg. were given. The implantations were spread over a period of 3 to 6 weeks in different experiments. Quinanil was added to prevent sepsis and litter mate controls were used in each case. The results have in all cases been treated statistically. The size of the testicles in the experimental animals was definitely less than that in the controls; there was no appreciable effect on the body weight. In one experiment 19 animals were implanted for 6 weeks. The average weight of the testicles at the end of the experiment was 0·488 g. as compared with 1·038 g. for a similar number of litter mate controls.

In all the animals there was a marked increase in the size of the suprarenals. In the series given above the suprarenals in the experimental animals averaged 0·025 gm. against 0·017 g. in the controls. This suggested some possible relationship between the suprarenals and the testicles, and implantation of adrenaline-free suprarenal tissue was found to inhibit the growth of the testicles; the suprarenals also became enlarged. Adrenaline-free cortical extracts (prepared by the method of Swingle and Pfiffner) also caused retardation of testicular growth with a very slight increase in suprarenal weight. Histological examination of the material is in progress.

The effect on certain endocrine organs of feeding with liver and with ventriculin after bleeding. By Esther A. Michael (*Physiology Dept., Univ. of Edinburgh.*)

Cats were rendered anæmic by repeated bleeding from the ear-vein. The animals were then divided into three batches, one to serve as control, one to be fed with raw liver and one with ventriculin (P.D. and Co.). They were kept on this diet for five weeks, then killed and certain of the endocrine organs removed and examined histologically and experimentally. Most of the sections were treated by ordinary routine stains. In addition some of those of the pituitary were stained with eosine and methylene blue [May and Grünwald] and thyroids with modified azan. The suprarenals were fixed in osmic vapour [Cramer's method].

Evidence was obtained that the anterior lobe of the pituitary, the thyroid and the suprarenal medulla are stimulated to increased activity by both liver and ventriculin. The anterior lobe of the pituitary showed an increase of basophils with a corresponding reduction of oxyphils, and these were smaller than normal. All the changes were better marked in the liver-fed animals than in those to which ventriculin was administered.

The vesicles of the thyroids of both liver and ventriculin-fed animals were smaller than normal and irregular in shape with a diminished amount of colloid. The lining epithelial cells were cubical or columnar. In the azan-stained specimens the colloid of the ventriculin group was stained in some cases blue and in others orange. There was more blue than in the control. In the liver group the colloid was mostly blue (indicating activity).

The suprarenal medulla of these animals showed definite changes. In the normal condition osmic stained adrenal medulla shows cells of two different tones of staining—one black and the other grey, both having clear turgid nuclei with fine greyish granules of adrenaline in the protoplasm. In the grey type in addition are found coarse black bodies—the *globoid bodies* of Cramer—considered by him to be either adrenaline or precursors of adrenaline. In the suprarenals of both ventriculin- and liver-fed groups the cells contain fewer granules and the nuclei of some are small staining dark brown. In these cells the protoplasm has a less granular appearance. Some of the cells are vacuolated. The capillaries are dilated. In the liver-group the blood in the veins contains greyish granules of adrenaline.

The adrenaline content of the gland was determined by bio-assay. It was found in all cases that for injections of similar amounts of adrenaline

(P.D. and Co.) and of the extracts of the suprarenals of the experimental animals there was a distinct increase of blood-pressure as compared with the control gland. The total adrenaline was also estimated chemically; the results corroborated those obtained by the histological and experimental methods.

The development of reactivity to gonadotropic hormones. By B. P. WIESNER. (*Macaulay Laboratory, Institute of Animal Genetics, Univ. of Edinburgh.*)

A number of female rats aged 0 to 2 days were injected with highly purified preparations of gonadotropic hormones. It was found that the ovary of newborn rats does not react to any perceptible degree to quantities sufficient to produce œstrus or even pseudopregnancy in rats 17 to 56 days old.

Similarly, it was found that differentiation of the uterus, which occurs during the first 14 days of life, is not accelerated by the administration of the œstrifying hormone. Reactivity to the œstrifying hormone develops gradually.

Effects of early oöphorectomy in rats. By B. P. WIESNER. (*Macaulay Laboratory, Institute of Animal Genetics, Univ. of Edinburgh.*)

Newborn female rats were oöphorectomized. A method used previously for the castration of males (immobilization by exposure to cold) was employed. The animals were operated on again at various ages and part of the uterus and vaginal wall was removed and kept for histological examination. The animals were then injected with gonadotropic hormones so as to detect any rest of ovarian tissue which hypertrophies after the administration of gonadotropic hormones. Cases in which it was detected, by this method, that oöphorectomy had been incomplete, were eliminated.

It was found that during the first 34 days of life differentiation and growth of the genital organs proceeds at a considerable rate. This differentiation is not impaired by oöphorectomy to any detectable degree.

The female rat differs thus from the male in which differentiation and growth of the genital organ is arrested by castration carried out immediately after birth.

The relation of the adrenals to anæsthetic hyperglycæmia.
By CHARLES REID.

The conclusion of Rogoff and Stewart [1920] that the adrenal epinephrin output is not essentially concerned in the hyperglycæmia induced by ether narcosis has been examined by three methods, viz. (1) direct, (2) comparative, (3) indirect.

(1) *Direct method.* This consists in determining by repeated tests the glycæmic response, *i.e.* the increase in blood sugar, induced in a series of 32 well-fed rabbits, starved for 12 hours, by maintaining surgical anæsthesia by open ether administration for 30 minutes, blood sugar being estimated before and after induction of narcosis and at the end of the 30 minutes' period.

Subsequently the left adrenal gland was removed or its medulla destroyed by aseptic technique through the lumbar route and the series of rabbits again subjected to the same procedures as regards anæsthesia and blood sampling.

After 2 to 4 weeks the right adrenal gland was removed or denervated and decapsulated or its medulla destroyed, and the effect of maintaining surgical anæsthesia on the blood-sugar level determined as before at intervals of several days following the second operation. Weight records were kept throughout.

During ether surgical anæsthesia for 30 minutes the glycæmic response was greatest in normal, less in single-operated and least in double-operated rabbits, *e.g.* increases of the order of 120, 100, 50 mg. p.c. respectively being obtained.

Operated rabbits are not perfectly normal animals. The absence of adrenaline or its immobilization by operation would prevent, during the stress of anæsthesia, the occurrence of adequate circulatory adjustments, in the absence of which the usual glycæmic response of the organism might be lacking, apart from the influence of a considerable decrease in the cortical tissue on the general well-being of the animal. This objection is partly met by data derived from the comparative and indirect methods.

(2) *Comparative method.* The red-brown Belgian hare type of rabbit is much less susceptible to adrenaline injections than the albino Himalayan type—as shown by the generally smaller glycæmic response of the former as compared with the latter (60 and 106 mg. p.c. respectively) to subcutaneous injections of adrenaline (0·15 mg. per kg. body weight). The anæsthetic glycæmic response of these groups differed similarly,

being 50 and 150 mg. p.c. respectively at the end of 30 minutes' surgical anæsthesia.

(3) *Indirect method.* This consisted in observing the glycæmic response to combinations of anæsthetics or narcotics. For example, morphine increased the blood sugar of rabbits and the body temperature rose, whereas amytal had little or no effect on the blood-sugar level but the body temperature fell. Morphine hyperglycæmia according to Rogoff and Stewart [1922] is dependent on intact adrenals and the centre controlling the exhaustion of adrenaline under anæsthesia is situated according to Elliott [1912] close to the bullar vaso-motor centres. A rabbit anæsthetized with amytal and tracheotomized shows a glycæmic response of the usual order to adrenaline (0·15 mg. per kg.) but little or none to the minimal effective dose of morphine. Similarly for ether, *e.g.* a rabbit or dog, amytalized first and given ether through a respiratory pump in the percentage necessary to produce anæsthesia in the non-amytalized animal, exhibits a much smaller and slower rise in blood sugar than the non-amytalized etherized animal. Amytal, therefore, would appear to depress the activity of the centre in the bulb controlling adrenal medullary secretion.

Summary. Evidence is adduced that the adrenals are concerned in anæsthetic hyperglycæmia.

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A varnish for smoked paper. By James R. Pirie.

(*Physiology Dept., Univ. of Edinburgh.*)

The following varnish for tracings on smoked paper gives an almost matt surface and does not cause the paper to become brittle or to scratch when dry. It is simply pure resin dissolved in pure rectified spirit. Many commercial resins are unsuitable owing to the presence of a vegetable mastic which leaves a white deposit on drying. Only pure amber resin should be used and each fresh supply tested for absence of the above white deposit before being employed.

The exact formula is:

Pure amber resin (powdered)	168 g.
Rectified spirit	2270 c.c.

Observations on the action of the respiratory centre.
By Grace Briscoe.

These observations were made in the course of some diaphragm-phrenic experiments in which one leaf of the diaphragm was treated as a peripheral preparation. The left phrenic had been cut in the neck (so that the chest was left intact) and respiration was carried on by the intercostal muscles and the right unparalysed side of the diaphragm. By appropriate stimulation of the cut peripheral phrenic [Briscoe, 1928] it was found possible to make the paralysed side of the diaphragm give curves of contraction and relaxation exactly similar to those on the unparalysed side, which was, of course, responding to impulses from the respiratory centre. As long as the modulation of the stimuli was done by hand (by moving a secondary coil to and fro close to a primary) it was found possible to reproduce natural contractions with very little disturbance of the intact side. As soon, however, as a mechanical oscillator was tried different results appeared. It was difficult to set the oscillator at exactly the same rate as the respiratory centre, and it was noticed that the phasic contractions induced on the paralysed side were having an influence on the phasic rhythm of the centre, as evidenced by the movements of the chest and normal side of the diaphragm.

Up to a point the experimental side could control the natural side, if there were not too much difference between the respective rhythms, and cause the centre to discharge at a rate which was other than its natural rate under existing experimental conditions. For instance: the respiratory centre working alone discharged every 2 sec., when the paralysed side was made to contract every $2\frac{1}{2}$ sec. the respiratory centre also took up this rate and returned to the 2-sec. interval when the stimulation was stopped. On shortening the experimental interval to $1\frac{3}{4}$ sec., the natural side was quickened for four beats, escaped with a couple of slow beats, then again four quick and two slow beats, so that a "pattern" appeared, repeating itself every six beats and corresponding to seven beats on the experimental side. When the experimental interval was further shortened to $1\frac{1}{4}$ sec. the natural side did not follow, but slowed to a rate which was half the experimental rate. The centre cannot be held long at a quickened rate, so that when the experimental rate is maintained slightly faster than the natural rate, the centre alternates between 1 to 1 and 1 to 2 ratios, with respiratory cycles of intermediate value in the transition stages.

If the natural rate be slow, and the experimental considerably faster, the central discharge can be further delayed and may even be prevented. Its onset, if delayed, is still influenced by the experimental contractions, as there exists under such circumstances a definite ratio between the contractions of the two halves of the diaphragm, *e.g.* 1 to 5 or 1 to 9.

Not only the time intervals of central discharge can be modified experimentally, but also the length of discharge, *i.e.* the time occupied by inspiration can be lengthened or shortened. An experimental contraction occurring after inspiration has begun shortens discharge, a relaxation occurring during inspiration prolongs discharge from the centre. These four modifications (delay or hastening of discharge, shortening or lengthening of inspiration) will produce patterns in the tracing from the natural side. Under certain conditions these patterns repeat themselves regularly. For instance, the centre was discharging regularly every 2·7 sec. The experimental side was set to contract every 1·8 sec., so that the sum of two natural intervals (5·4 sec.) corresponded to the sum of three experimental intervals. The natural side now discharged at irregular intervals, 2·45, 2·95 sec., but these irregularities were repeated regularly every 5·4 sec.

When patterns repeat, but not quite regularly, it is found that the period occupied by the whole pattern does not coincide closely with a whole number of natural intervals. For instance, a pattern of five irregular intervals (2·94, 4·1, 3·1, 3·08, 2·87 = 16·39 sec.) coincided with six experimental intervals of 2·73 sec. each = 16·38 sec. It did not coincide with any whole number of natural unmodified intervals, and the repetition was not exact.

Section of the vagus on the experimental side while modification is in progress may stop the induced effects at once. After a pause distinct control effects can be obtained, even with both vagi cut, though not with such ease as before section.

Control effects are more easily obtained under deep anæsthesia.

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STUDIES CONCERNING THE ALIMENTARY ABSORPTION OF WATER AND TISSUE HYDRATION IN RELATION TO DIURESIS.

By H. HELLER and F. H. SMIRK[1].

(From the Pharmacological Institute, University of Vienna and the Medical Unit, University College Hospital Medical School.)

Our primary interest in this work has been the concurrent study of water diuresis and water storage in tissues both normally and under experimental conditions which inhibit the formation of urine, and it will be the object of these papers to show how numerous and interrelated are the factors which may modify or prevent the response of the kidneys to water administered by stomach tube.

To this end we have tried to determine how a dose of water received by the alimentary tract is normally distributed about the body and how the distribution is altered by measures which reduce the urinary output.

The disposal of this additional water throughout the animal body may be represented diagrammatically as follows:

$$\begin{tikzcd} & \text{Alimentary tract} \arrow[d, shift left] & \text{Extrarenal loss (respiratory sweating)} \\ \text{Tissues} \arrow[r, shift right] & \text{Plasma} \arrow[l, shift right] \arrow[u, shift left] \arrow[d, shift left] \arrow[ur] \arrow[dr] & \\ & \text{Corpuscles} \arrow[u, shift left] & \text{Kidney} \end{tikzcd}$$

For clarity we have subdivided our investigations of the above system.

Part I. The alimentary absorption of water in the rabbit, guinea-pig and rat takes place at different rates and, from this alone, differences in the responses of the kidney to water administration would be expected. Not only is this so but we find there is no parallelism between the load of absorbed but unexcreted water and the rate of urine formation at a given moment.

Although the times and rates of absorption and diuresis and the time relationships between them vary with the species of animal they appear to be fairly constant for any one species.

[1] Beit Memorial Research Fellow.

Our experiments have been made on the rabbit, rat and guinea-pig, and from indirect evidence which will be presented by one of us at a later date it seems that the absorption-diuresis relations in man bear most resemblance to those of the rat.

In the rat absorption from the alimentary canal may be studied readily by a statistical method; sets of rats being killed at varying times after water administration and the average amount of absorbed water determined by weighing the gut. In the guinea-pig this method of study is possible but less accurate because of errors introduced by the larger amount of residue in the lumen of the alimentary canal, and in the rabbit for this same reason it would be even harder to obtain reliable results.

In the rabbit therefore we have studied water absorption indirectly by determining the increased water content of blood, muscle and skin. The sources of error incidental to such a method have been controlled as far as possible.

Part II. The amounts of water lost extrarenally by small animals is large relative to their respective weights. Usually the loss takes place by the respiratory tract. A rat may lose as much as 1 p.c. of its body weight per hour and in a warm environment more is lost.

Now in Part I it is shown that the rate of urine formation is not parallel to the load of absorbed but unexcreted water in the body; nor is it parallel to the degree of plasma or whole blood dilution [Smirk, 1932]. Since diuresis is neither proportional to the degree of blood dilution nor to the excess of absorbed water in the body at any given time, it becomes a matter of some interest to know how extrarenal losses of water affect the urinary output.

We have been able to show that rats and rabbits when living in a warm environment may lose as much as 4 or 6 p.c. of their body weight in a relatively short time. So that if these animals are depleted of water and then are given 4 or 5 p.c. of their original body weight of water they may have, compared with their resting condition, only a slight excess of water in the body (as in the rabbits) or even a deficit of water (as in the rats). Nevertheless in both rats and rabbits the diuresis which follows this new supply of water is not very much less when the animal has been depleted of water previously, than in control experiments upon the same animals where there has been no preliminary water depletion and any additional water given constitutes an excess. Diuresis may apparently occur when there is no excess of water in the body. This leads us to a point of clinical interest.

The reduced urinary output of febrile patients is usually considered

to be the result of increased extrarenal loss of water. In rabbits and rats considerable extrarenal loss of water did not greatly reduce the urinary output and it appeared a suitable opportunity for testing whether an increase in temperature would produce oliguria in animals as in man and whether this reduction in urinary output might be in part due to some effect of pyrexia other than increased extrarenal loss.

The posture of an unanæsthetized animal makes a great difference to its powers of thermo-regulation and secondarily to its urinary output. This subject—of general importance in experimental work—is briefly dealt with.

Part III. In Part I it was found necessary to have a control observation upon the increase in the water content of muscle when water was absorbed normally but was not excreted by the kidneys. For this purpose use was made of the inhibitory action of pituitrin upon water diuresis. It was demonstrated that pituitrin did not cause any significant diminution in alimentary water absorption in the rat, and the presence in tissues of absorbed but unexcreted water was clearly demonstrated in the rabbit.

Although these results were originally obtained for another purpose they appear to provide additional evidence that the anti-diuretic action of the pituitary hormone is of primarily renal origin. The distribution of water in the blood tissues and serous cavities makes it unlikely that the pituitary hormone operates through any general change in the physico-chemical states of membranes or tissue proteins. For this reason the pituitary work has been treated separately.

Part IV. In clinical surgery and in experimental physiology it may be observed that the urinary output is changed by anæsthesia and laparotomy. This influence of anæsthetics upon diuresis is naturally of importance in the control of any renal experiments for which an anæsthetic is required, and the matter is interesting in relation to the nervous control of water metabolism.

From the school of Pick numerous papers [Kugel, 1929; Bonsmann, 1930 and Truemann, 1931, etc.] have been published which indicate that most anæsthetic substances inhibit a diuresis produced by the alimentary administration of water.

The influence of an animal's water absorption rate upon its urinary output (to be described in Part I) raises the question of whether anæsthetics may influence diuresis by delaying absorption or whether the anæsthetic acts upon an extra alimentary system, its effect being manifested after absorption has taken place. In regard to this last point Hicks and Smirk [1930] have demonstrated that when a blood dilution

has been produced by administration of water to rabbits the subsequent administration of chloretone causes a reconcentration of the blood and diminution of the urinary output.

Part I. The absorption and distribution of water during normal water diuresis in rabbits, rats and guinea-pigs.

INTRODUCTION.

The determination of the normal relationships between the absorption of water in the alimentary tract, its storage in blood and tissues and its subsequent excretion by the kidneys is made difficult by the probability that changes in all three factors may well occur as a result of the experimental procedures necessary in their investigation. Particularly is this so where general anæsthetics are used. Thus ether, chloralose, chloretone, chloralhydrate, morphia, isopral, veronal, luminal and trional have been recorded as inhibitors of water diuresis; urethane, paraldehyde and veronal have been stated to increase the urinary output. The extensive and in part contradictory literature is briefly summarized by Kugel [1929], and it would seem that although the results obtained depend on the animal used, the depth of narcosis and the length of the experimental period, there is no doubt that ordinary narcotic substances produce marked alterations in the amount of urine excreted and are clearly unsuitable for the investigation of its normal progress.

Decerebrate or decorticate dogs not under the influence of morphia and allowed to recover for one or more hours from the volatile anæsthetic have been used in investigations of this type by Fee [1929], Bayliss and Fee [1930].

They obtained a water diuresis which appeared to follow the normal course and investigated the changes in blood concentration. This method has the advantage that the effects of emotional disturbances and of muscular exertion on water metabolism are avoided. In water-diuresis tests on rabbits performed before and not less than 12 hours after the use of any general anæsthetic Godlowski [1930] finds an increased urinary output after decortication and after decerebration, either an increase or decrease depending, it appears, upon the level of section. We have preferred, however, to use local anæsthesia for the removal of our tissue samples because in the investigation of a process perhaps intimately bound up with the nervous system we hesitated to begin our investigations

by removing an important part of it. There remains with us the difficulty which Fee eliminated, namely, the effect of emotional disturbance in the animal on the course of diuresis. We have attempted to minimize this and to demonstrate by control observations in what degree and directions reliance may be placed on our results.

A. Rabbits.

Experimental procedures.

The removal and analysis of blood samples.

Blood samples were obtained from a marginal ear vein in rabbits, and heparin was our usual anti-coagulant *in vitro*. The blood dilution was investigated by several methods. The total solids of whole blood were determined by drying to constant weight at 107° C. The hæmoglobin was determined by conversion of a measured volume of blood to carboxy-hæmoglobin and colorimetric comparison of the samples taken before and after water administration. The plasma proteins were determined by Kjeldahl's method. The samples of blood removed were usually 0·5 c.c. The loss of blood during removal of muscle samples was slight. Probably the total loss before taking the second sample would not be more than 0·8 c.c. where muscle and blood samples alone were taken. The removal of 0·8 c.c. of blood does not appreciably affect the dilution of the second sample [Smirk, 1932].

The removal of muscle samples.

Two muscle samples were removed from symmetrical portions of the longissimus dorsi on either side, one before and one usually 2 hours after water administration. Local skin anæsthesia was maintained by 2 p.c. novocaine and 0·0004 p.c. adrenaline—the muscle not being infiltrated. The fibres of a suitable strip of muscle (1·5 × 0·5 cm.) are split longitudinally by a sharp scalpel, and the piece between may then be excised by short snips with a pair of scissors. The rabbit sits in a box and need not be held during the slight operation. The small wound is plugged with wool soaked in 2 p.c. novocaine to maintain anæsthesia and the animal is killed at the end of the experiment. The water content of the muscle is determined by drying to a constant weight at 107° C. The weight average of the muscle samples is about 0·8 g. A second muscle sample is obtained by the same procedure. In a few of the experiments performed in Vienna the wound was kept sterile, the animal preserved for a few days and an experiment repeated.

Control on the effect of local anæsthetics on a water diuresis.

Five rabbits received 2 c.c. of a solution containing 2 p.c. novocaine and 0·0004 p.c. adrenaline subcutaneously, 15 min. before the administration of 40 c.c. of water per kg. of body weight and then 4 c.c. of the novocaine-adrenaline solution 1½ hours after water administration. The amounts of urine formed in the 3 hours after water was given were 87, 77, 73, 71 c.c. (average 78·1).

In a set of experiments on the same rabbits where no local anæsthetic was given the corresponding outputs of urine were 95, 37, 102, 66, 75 c.c. (average 75·0).

Two rabbits received each 3 c.c. of 4 p.c. novocaine intraperitoneally and 1 hour later 72 c.c. of water by stomach tube. The amounts of urine over a period of 150 min. were 32 c.c. and 53 c.c. as compared with control diuresis tests in the same animals of 40 c.c. and 51 c.c.

In two other rabbits intraperitoneal injections of novocaine and adrenaline did not produce any significant change in urinary output.

There is no difference in urinary output which can be attributed to the use of local anæsthetics.

Control on the effect of the removal of blood samples from the ear veins, and of muscle samples from the longissimus dorsi on a water diuresis.

In a series of twenty-six experiments on thirteen different rabbits the diuresis following the administration of 40 c.c. of water per kg. body weight did not appear to be altered significantly by the procedures of removing blood and muscle samples, and in eight of these in which the

TABLE I.

Animal No.	Control—urine samples only		Blood and muscle samples taken (animal unbound)	
	Urine in 3 hours c.c.	Temperature change in ° C.	Urine in 3 hours c.c.	Temperature change in ° C.
17	60	-0·5	59	-0·2
16	64	-0·2	53	-0·0
15	43	-0·0	69	-0·1
10	51	-0·3	41	-0·1
11	20	-0·1	39	+0·5
12	34	-0·2	45	+0·4
13	29	-0·3	15	-0·1
14	30	-0·2	13	-0·0
18	35	—	29	—
19	67	—	121	—
20	5	—	53	—
21	22	—	20	—
22	20	—	21	—
Average	37		44	

rectal temperature was measured there was no change worthy of special note. The actual figures are given in Table I.

The average output of urine from the thirteen animals was 44 c.c. when muscle and blood samples were taken and 37 c.c. in the preliminary diuresis. It would therefore appear justifiable to consider that the uptake of water from the alimentary canal and the subsequent urinary output are not disturbed by taking samples of blood and muscle.

Control observations on the water content of muscle.

(1) Variations in the water content of the longissimus dorsi muscle from different batches of animals living under different conditions; and individual members of the same batch living under the same conditions (no additional water given).

TABLE II.

	Average water content p.c.	Water content of the muscle samples from individual rabbits p.c.
Batch I (5 rabbits)	74·8	75·0; 74·5; 74·5; 74·9; 74·9
Batch II (3 rabbits)	76·8	76·9; 75·8; 77·9
Batch III (2 rabbits)	—	75·8; 77·7
Batch IV (5 rabbits)	76·1	76·6; 76·1; 76·2; 76·1; 75·3
Batch V (2 rabbits)	—	77·7; 76·6
Batch VI (5 rabbits)	75·7	74·1; 75·8; 75·7; 76·3; 76·6
Batch VII (4 rabbits)	77·1	75·9; 78·2; 76·7; 77·7

It will be seen that as a rule the variations between batches of animals living under different conditions are rather greater than between members of the same batch. The members of the various batches of rabbits were not necessarily or usually of the same litter but were similarly housed and fed.

There did not appear to be any constant difference in the degree of muscle hydration between groups fed on oats and groups fed on cabbage.

(2) Variations in the water content of longissimus dorsi muscle taken from the same animals at an interval of 2 or 3 days (no additional water given).

It will be seen that at different times variations of muscle hydration are observed in animals which have at all times been allowed free access to food and water.

TABLE III.

		Percentage water content of the muscle samples from rabbit				
No. of animal ...		1	2	3	4	5
Batch I (5 animals)	1st sample	75·9	78·2	76·7	77·7	77·2
	2nd sample	75·9	78·3	76·6	79·3	77·1
		6	7	8	9	
Batch II (4 animals)	1st sample	76·6	76·1	76·2	76·1	
	2nd sample	75·0	74·5	74·5	74·9	
		10	11	12		
Batch III (3 animals)	1st sample	75·3	77·7	76·6		
	2nd sample	74·9	75·8	77·9		

(3) The constancy of longissimus dorsi muscle samples taken at nearly the same time from symmetrical parts on either side (no additional water given).

TABLE IV.

	Percentage water content of muscle samples from individual rabbits				
No. of animal ...	6	5	13	14	3
Sample from right side	76·6	77·1	77·8	76·9	76·6
Sample from left side	76·4	77·1	77·7	77·0	76·5

Symmetrical parts of the same muscles at the same time have equal water contents.

(4) Variations in the water content of muscle samples from symmetrical parts of the longissimus dorsi and vastus externus taken at intervals of 2 hours (no additional water).

TABLE V.

		Percentage water content of muscle samples from individual rabbits		
No. of animal		15	16	17
Longissimus dorsi	1st sample	77·4	77·2	76·4
	2nd sample	78·4	76·7	76·1
Vastus externus	1st sample	79·5	79·2	78·3
	2nd sample	79·3	77·8	77·4

As would be expected there is a tendency for the water content of muscle to be diminished slightly over a period of 2 hours owing to loss of water by the renal and respiratory systems. In all samples the water content of vastus externus has been greater than that of longissimus dorsi. The changes in water content are not equal.

(5) The detection of the additional water in muscles when its excretion by the kidneys is prevented: the instance chosen being the inhibition of diuresis by pituitary hormone.

It will be shown in Part III that although the uptake of water is not equal in all muscles yet there is no doubt that when water is absorbed but not excreted by the kidneys there is a substantial increase in the water content of muscle, often as much as 2 or 3 p.c.

The storage of water in muscle and blood during a normal water diuresis.

The following table gives the changes in blood concentration and in the water content of muscle samples taken before and after the administration of 40 c.c. of lukewarm tap water per kg. body weight to rabbits by means of a stomach tube. The resulting urinary outputs have been given in Table I, where they are compared with a previous set of experiments in which water was given but no blood or muscle samples were taken. It was shown that these additional procedures had no significant influence on the diuresis.

TABLE VI.

Animal No.	Blood concentration following water expressed as a percentage of the original concentration. Time after water administration: 30 min.	90 min.	120 min.	Methods of determining the water content of blood	Water content of muscle in g. water per 100 g. muscle: Before water given	Time after water administration: 90 min.	120 min.	Difference in water content p.c.
17	95·0	96·8	—	By percentage of solids in blood	—	—	—	—
16	97·5	94·2	—		76·6	76·6	—	+0·0
15	96·0	91·4	—		77·7	78·1	—	+0·4
10	—	93·8	—		76·6	76·6	—	+0·0
11	—	106·0	—		76·1	76·1	—	+0·0
12	—	96·3	—		76·2	76·6	—	+0·4
13	—	95·2	—		76·1	75·0	—	−0·1
14	—	104·0	—		75·3	75·7	—	+0·4
18	—	—	91·4	By dilution of proteins det. by Kjeldahl's method	74·1	—	74·2	+0·1
19	—	—	97·0		75·8	—	76·3	+0·5
20	—	—	—	—	76·3	—	76·7	+0·4
21	—	—	—	—	76·6	—	78·1	+1·5
22	—	—	—	—	75·7	—	76·4	+0·7
								Av. +0·33

It will be seen from Table VI that the degree of blood dilution varies considerably from one experiment to another, but the most general figure obtained is a reduction in the concentration of solids to 95 p.c. of their amount in the undiluted blood. Twice there was no dilution and the blood appeared to be more concentrated. Methodical error is unlikely and there are numerous difficulties attached to the interpretation of blood-dilution experiments such as the changes due to splenic contraction, changes in blood concentration following the muscular exercise of struggling or following alterations in blood-pressure.

In view of the possible difficulties introduced by slight losses of blood during the removal of muscle samples and the many conflicting statements in the literature concerning the presence or absence of a blood dilution in the course of water diuresis, a short separate study of this matter has been made by one of us [Smirk, 1932]. It was shown that in rabbits a whole blood dilution of 2 or 3 p.c. is usual, and that the larger part of this additional water is held by the plasma.

It will be observed from Table VI that the uptake of water in muscle is slight, and the total quantity of water represented by this in the animal's body is not great. The average change in the water content of the muscles is 0·33 p.c.

The average percentage weight of the striated muscle and of the skin in rabbits.

For further calculations it is of importance to know the relative amounts of muscle and skin in rabbits. In a series of nine animals the striated muscle accounted for approximately 40 p.c. and the skin with fur 12 p.c. of the total live weight. It is probably fair to assume another 5 or 8 p.c. for unstriated muscle, so that the weight of muscle in a 2000 g. rabbit will be about 900–950 g. Since most of our rabbits weigh 2000 g. and we may assume approximately 1000 g. of muscle with an average water percentage of 75 this mean change of 0·33 p.c. represents about 13 c.c. of additional water. In only two of the thirteen experiments was the water increase in muscle as much as 0·6 p.c. at a period of 1½–2 hours after water administration.

It must be remembered that if the above calculation is applied to individual muscle samples instead of to representative averages it will give only approximate indications of the water stored in muscle.

The storage of water in skin during a normal water diuresis.

In a short series of five animals small samples of skin (+ hair) were removed under local subcutaneous anæsthesia. The samples were cleaned of subcutaneous tissue and their water content was determined before, and 90 min. after, water administration by the method described for muscle.

The uptake of water was rather variable and appeared to be somewhat greater than that of muscle. The actual increases in water content were + 3·5, + 1·1, − 1·6, + 0·6, + 5·5 p.c. (average 1·8).

The necessity for local anæsthesia introduces a source of error.

During the normal diuresis in rabbits there does not appear to be any great increase in the water content of the muscles. Occasionally rabbits

are met where the normal output of urine in response to water administration is very low, yet the increase in the water content of muscles is as a rule no greater in these animals than in those where the output of urine is much greater (see Tables I and VI).

It will be shown later (Part III) that when diuresis is prevented or reduced by pituitary hormone a considerable increase in the water content of the muscle is obtained. It would appear from this that so long as the water given has been absorbed and is not yet excreted there will be a definite increase in the water content of muscle—say 2 or 3 p.c. The absence of such an increase in rabbits where the diuresis is poor is strong presumptive evidence that the water has not been absorbed. A possible alternative explanation would be that pituitrin might increase the absorption of water, and muscle hydration result as much from an increase in the uptake as from the diminished output. There is, however, no evidence in favour of this hypothesis, and in rats under the influence of pituitrin the water absorption curve is practically identical with the normal (see Part III).

The excess of water which is stored in muscle and blood prior to excretion is not often more than 20 c.c. when 80 c.c. have been given, and it appears in the rabbit that the excretion rate may lag only a little behind the alimentary absorption rate.

As the absorbed water is largely stored in the muscles when the normal renal response is inhibited by pituitrin it is of interest to know if the same relations between absorption and excretion hold in animals where water absorption takes place more rapidly. We therefore decided to study the diuresis in relation to the water absorption from the alimentary canal in rats and guinea-pigs.

B. Rats.

Since the rat is a smaller and less docile animal it would not be easy to obtain muscle samples and use their water content as an index of water storage in tissues. For the direct study of alimentary water absorption however the rat is much more suitable than the rabbit, and if in addition the urinary output is known we can deduce whether there is any appreciable storage of water in tissues as a normal phenomenon of water diuresis.

The method of study we have selected is as follows. A rat is weighed. 5 p.c. of its body weight of warm water is administered by a small stomach tube (No. 8 catheter). The animal is returned to its cage and at a selected

time after giving the water it is killed by a blow on the head and the alimentary canal is dissected out and weighed together with its contents. The longer the waiting period after water administration the more water is absorbed, and with a sufficiently large number of rats killed at varying times after giving water a normal average absorption curve may be constructed.

If now a series of similarly treated rats are also given 5 p.c. of their body weights of water, an average of their urinary outputs may be taken as a normal average curve of diuresis corresponding with the average absorption curve already obtained. The urine of individual rats can be collected by keeping the animals in small wire cages. On shaking the cage or taking hold of the animal's tail it voids urine even if only a little has been formed. The effectiveness of this method has been demonstrated by subsequent examination of the urinary bladder, so that by this means it is possible to obtain the precise onset of diuresis. The urine is collected on cotton-wool swabs and weighed.

Results.

The investigations on water absorption are summarized in Tables VII and VIII, from which may be constructed the two water-absorption curves of Fig. 1. The first gives the rate of water absorption in

TABLE VII. Animals allowed free access to water.

Time after giving water (min.)	Number of rats used	Weight of the alimentary canals and contents expressed as a percentage of body weight	Average
20	5	8·9; 8·6; 7·9; 7·8; 9·2	8·5
30	4	9·3; 8·2; 7·8; 8·0	7·7
	4	6·6; 7·1; 6·4; 7·9	
45	6	5·5; 6·5; 6·6; 6·5; 6·1; 5·9	6·2
60	5	6·6; 6·2; 7·1; 6·2; 6·2	6·5
75	7	4·6; 10·3; 6·4; 7·8; 5·9; 5·5; 5·0	6·5
120	6	6·8; 6 2; 7·7; 7·6; 5·9; 4·8	6·5
Controls, no extra water given	10	6·0; 5·9; 6·1; 5·0; 5·1; 5·1; 6·2; 5·7; 5·2; 5·0	5·5

TABLE VIII. Animals deprived of water overnight.

Time after giving water (min.)	Number of rats used	Weight of the alimentary canals and contents expressed as a percentage of body weight	Average
15	3	8·0; 8·4; 9·2	8·5
30	9	9·60; 6·5; 4·8; 6·9 (av. 6·9)	6·8
		6·4; 6·8; 7·7; 6·2; 6·5 (av. 6·7)	
45	7	6·9; 6·3; 6·4; 6·1; 6·1; 5·7; 5·7	6·2
Controls, no extra water given	7	5·3; 5·8; 6·7; 6·8; 7·0; 6·58; 6·20	6·3

animals allowed free access to water up to the time of the experiment, and the second gives the rate in animals deprived of water overnight. The percentage weight of the gut was 0·75 higher in the animals deprived of water (perhaps owing to their previous diet), and in order to render the two curves more easily comparable 0·75 was deducted from all the average values on Table VIII before graphing the results.

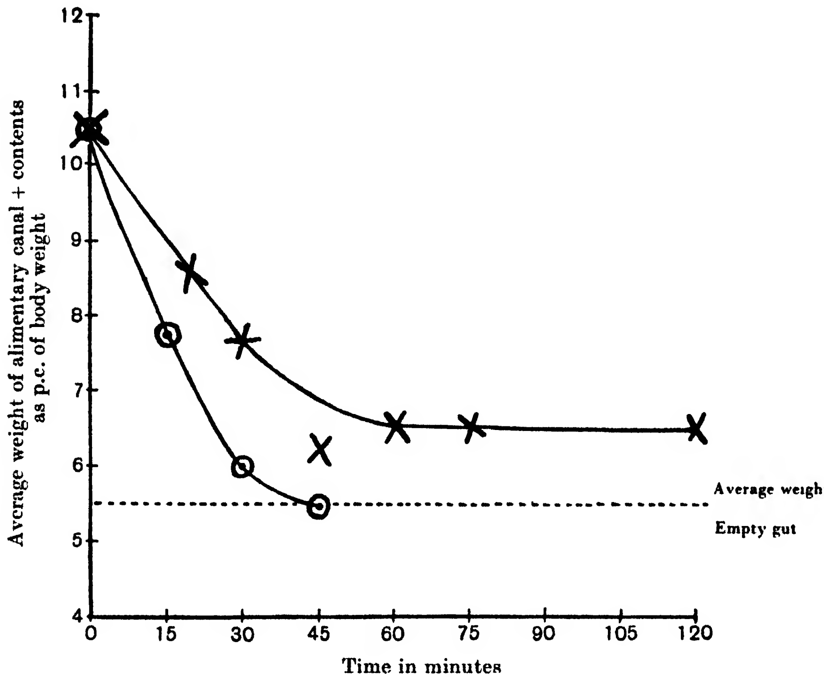

Fig. 1. Average water-absorption curves of rats: (*a*) previously allowed free access to water —×—×—, (*b*) previously deprived of water overnight —⊙—⊙—. The average weight of the empty gut is 5·5 p.c. of the body weight and 5 p.c. of the body weight of additional water was given, making 10·5 p.c. the starting-point of the absorption curves.

It would appear that water absorption proceeds more slowly in animals allowed free access to water up to the time of the experiment than in animals previously deprived of water overnight. We do not as yet regard this difference in absorption rate as sufficiently proved, but consider that, firmly established, it would be relevant to the problem of the physiological regulation of intestinal absorption.

In eighteen rats the diureses in response to 5 p.c. of their body weights

Table IX. Total output of urine expressed as c.c. per 100 g. rat.

No. of Animal	Time after water administration (min.)* 0	20	40	60	80	100	120	140	160	180
1	0·00	0·01	0·25	1·90	2·55	2·75	2·86	2·94	2·96	3·00
2	0·00	0·14	0·50	0·95	1·72	2·20	2·30	2·32	2·35	2·35
3	0·00	0·50	2·04	3·40	4·05	4·17	4·27	4·35	4·42	4·50
4	0·00	0·04	0·10	0·23	1·10	2·05	2·37	2·66	2·95	3·25
5	0·00	0·07	0·20	0·42	1·37	1·85	2·00	2·05	2·10	2·15
6	0·00	0·15	0·65	1·12	1·83	1·87	1·93	1·97	2·00	2·04
7	0·00	—	1·40	3·85	4·10	4·15	4·20	—	—	—
8	0·00	—	0·85	1·95	3·20	3·80	4·00	—	—	—
9	0·00	—	1·00	2·45	3·15	3·45	3·60	—	—	—
10	0·00	—	0·95	2·00	2·60	2·70	2·75	—	—	—
11	0·00	—	1·30	2·45	2·75	2·85	2·85	—	—	—
12	0·00	0·35	1·40	2·80	3·40	3·65	3·75	3·80	3·80	3·80
13	0·00	0·00	0·40	1·05	2·05	3·00	3·10	3·10	3·10	3·10
14	0·00	0·20	0·70	1·75	2·70	3·30	3·35	3·35	3·35	3·35
15	0·00	0·10	0·80	2·00	2·85	2·95	2·95	2·95	2·95	2·95
16	0·00	0·70	1·60	2·80	3·95	4·10	4·15	4·15	4·15	4·15
17	0·00	0·60	1·95	3·35	3·75	3·75	3·75	3·75	3·75	3·75
18	0·00	0·20	0·55	1·25	2·70	3·55	3·60	3·60	3·60	3·60
Average	0·00	0·23	0·93	1·95	2·75	3·10	—	—	—	3·35

* When urine samples were not obtained at the correct time the output for that time was determined graphically.

of water have been studied (Table IX), the preceding dietetic condition and allowance of water being similar to that of the animals of Table VIII used for the determination of absorption rates. The urinary outputs were expressed as c.c. urine per 100 g. rat. From these an average curve (not given) of the total urinary output was constructed expressing the results as c.c. of urine per 100 g. rat. From this curve the average rate of urine secretion for each 10-min. interval was determined in c.c. urine for 100 g. rat per hour (Fig. 2). Also on Fig. 2 are included two other curves. Firstly, the corresponding average water-absorption curve derived from Fig. 1, and secondly, a graph representing the average amount of absorbed but as yet unexcreted water in the body at any given time, which is obtained by subtracting the average total output of urine from the average volume of water absorbed at any given time and correcting for the average extrarenal loss of water (Part II).

It will be seen, on comparing the water absorption with the diuresis curve, that water absorption is well advanced before any notable increase takes place in the formation of urine. This is despite the fact that the dose of water is large: its equivalent would be 4 litres for an 80 kg. man but only 700 c.c. if the comparison were made by Rubner's surface formula. Also water absorption is practically complete some 15 or 20 min. before the height of diuresis is attained.

This lack of parallelism between water absorption and diuresis is further exemplified by the water-load curve. The average maximum load of absorbed but as yet unexcreted water is present in the rat 30 min. after giving the water. The rate of urine formation at this time is 1·2 c.c. per hour per 100 g. rat. It is another 30 min. before the kidney attains its maximum rate of urine formation.

Again, at the time when the rate of urine formation is greatest the load of absorbed water is 2 c.c. It also averages 2 c.c. ¼ hr. after giving

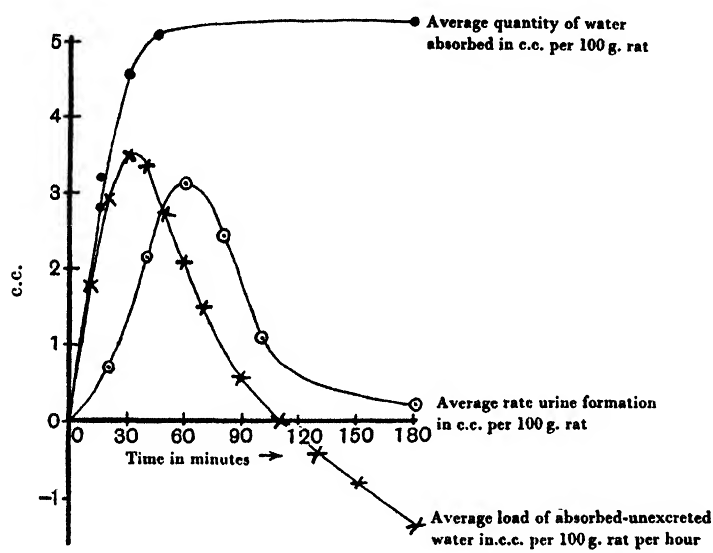

Fig. 2. The relationship between alimentary absorption, the water load and the rate of urine formation in rats. The water load is corrected for extrarenal losses of water from the respiratory tract.

water before absorption is complete, but in contrast at this time the rate of urine formation is only 0·3 c.c. per hour per 100 g. rat. With the same water-load distributed between the blood and tissues of the body the urinary outputs are regularly very different, being greater on the fall than on the rise of the water-load curve. Thus, when the water load is low shortly after giving the water and again as diuresis is subsiding, for equal water loads the urinary outputs are very different.

Since the uptake of water from the alimentary tract is clearly well in advance of diuresis it should be possible to demonstrate the increased water content of the body tissues which we have deduced, these increases being indicated approximately by the water-load curve. To obtain direct

confirmation of this samples of muscle and liver were removed from rats killed 20, 30 and 40 min. after the administration of water and, as controls, from rats in other ways similarly treated but to which no additional water had been given. A comparison of the water contents of the livers and muscles is given in Tables X and XI and in Fig. 3.

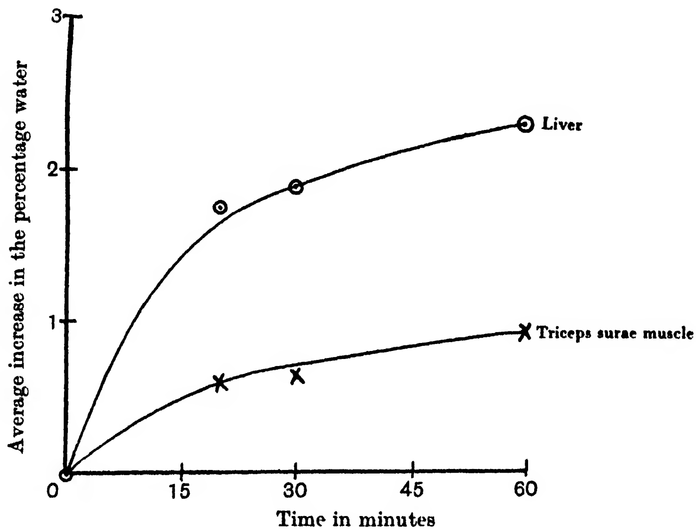

Fig. 3. The average water uptake in the liver and muscle of the rat following the administration of 5 p.c. of the body weight of water by mouth.

TABLE X. The water content of liver before and after administration of water. (Rats.)

Time after giving water (min.)	Controls (no water given) solids p.c.	No. of controls	Average of controls	Experiments (water given) solids p.c.	No. of exps.	Average of exps.	Difference in water content
20	28·4; 28·8; 28·8; 26·8; 29·3	5	28·44	26·4; 24·2; 26·6; 27·7; 28·2	5	26·6	+1·77
	28·0; 28·9; 28·9; 28·6	4	28·60	25·9; 25·8; 27·2; 27·2	4	26·52	+2·08
30	30·8; 32·2; 28·9; 30·6	4	30·62	29·8; 28·7; 29·6; 29·2	4	29·32	+1·3
	28·0; 28·9; 28·9; 28·6; 28·3; 28·9; 27·8; 28·1	8	28·44	25·9; 25·8; 27·2; 27·2; 26·9; 28·3	6	26·88	+1·56
60	28·3; 28·9; 27·8; 28·1	4	28·3	25·3; 25·5; 27·4; 24·9; 25·8	5	25·8	+2·5
	28·0; 28·9; 28·9; 28·6; 28·3; 28·9; 27·8; 28·1	8	28·44	27·4; 25·3; 25·5; 24·9; 25·8; 27·6; 27·7	7	26·31	+2·13

TABLE XI. The water content of muscle before and after administration of water. (Rats.)

Time after giving water (min.)	Controls (no water given) solids p.c.	No. of controls	Average of controls	Experiments (water given) solids p.c.	No. of exps.	Average of exps.	Difference in water content
20	23·4; 23·3; 24·0; 24·8; 24·1	5	23·9	23·8; 22·8; 24·1; 22·7; 23·1	5	23·3	+0·61
30	24·9; 25·4; 24·0; 24·3	4	24·65	24·3; 24·1; 23·2; 24·3	4	23·97	+0·68
	27·0; 27·6; 26·9; 26·7	4	27·05	26·8; 24·6; 26·9; 25·6	4	25·98	+1·07
	24·9; 25·4; 24·0; 24·3; 25·5; 24·8; 24·2	7	24·73	24·3; 24·1; 23·2; 24·3; 24·3; 24·8	6	24·17	+0·56
60	25·5; 24·8; 24·2	3	24·83	23·5; 24·0; 24·1; 24·1; 23·7	5	23·88	+0·95
	24·9; 25·4; 24·0; 24·3; 25·5; 24·8; 24·2	7	24·71	23·5; 24·0; 23·5; 24·1; 23·7; 23·5; 24·1	7	23·77	+0·94

It will be seen that there is a significantly greater water content in the tissues of the animals which received water. Particularly is this so in the case of the liver.

C. GUINEA-PIGS.

The experiments relating water absorption to diuresis have been repeated on guinea-pigs using the technique already described for rats. The study of water absorption is however more difficult in the guinea-pig, since the weight of the alimentary canal and its contents are usually 20 p.c. of the total body weight, whereas in the rat they are 5 or 6 p.c. The addition of 5 p.c. of the body weight of water will therefore make a smaller proportional difference to the gut weight in the guinea-pig, and its absorption is less easily followed by statistical methods.

TABLE XII.

Time after giving water (min.)	Number of guinea-pigs used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
15	6	26·7; 21·7; 26·4; 34·3; 26·0; 24·6	26·2
60	20	27·4; 18·8; 24·9; 18·7; 24·5; 24·0; 21·8; 18·4; 21·2; 24·7; 19·0; 20·8	22·0
		20·3; 14·9; 18·2; 16·6; 18·0; 15·3; 13·0; 19·9	17·2*
Controls, no extra water given	18	22·2; 18·8; 23·8; 14·9; 22·5; 24·7; 21·7; 26·2; 20·2; 19·2; 16·8; 21·0	21·0
		11·5; 18·5; 15·3; 14·0; 17·5; 20·0	16·1*

* These two groups of guinea-pigs were of a different breed and were differently fed and housed.

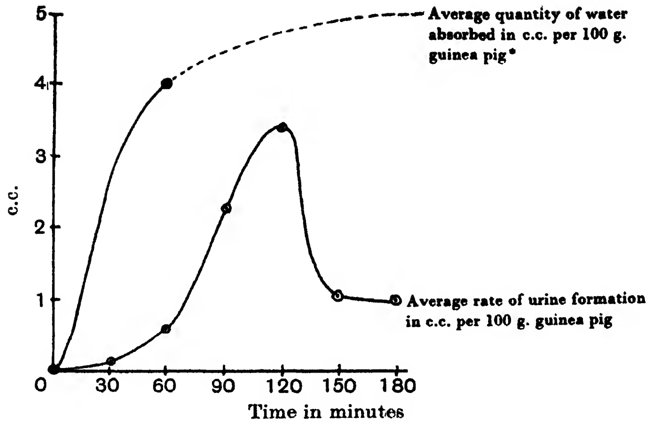

Fig. 4. The relationship between alimentary absorption and the rate of urine formation in guinea-pigs.

* The dotted line extension is presumptive, from analogy with the rat.

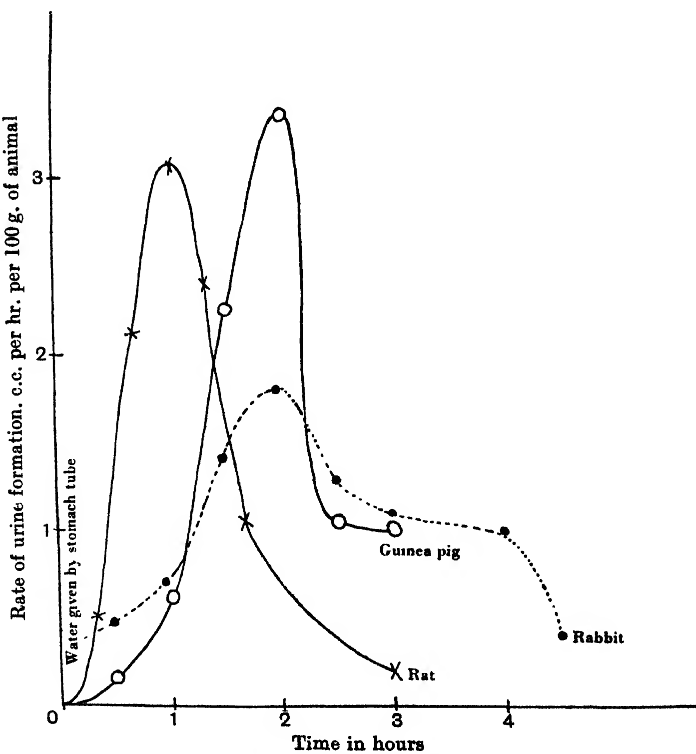

Fig. 5. The diuresis of the rat, guinea-pig and rabbit.

TABLE XIII.

Time after water administration (min.)	Total output of urine expressed as c.c. per 100 g. guinea-pig					
	Animal 1	2	3	4	5	Average
0	0·00	0·00	0·00	0·00	0·00	0·00
30	0·00	0·20	0·00	0·10	0·00	0·06
60	0·65	0·75	0·00	0·25	0·25	0·38
90	1·60	1·35	0·40	0·95	3·60	1·58
120	2·60	3·20	2·82	2·50	4·75	3·17
150	3·20	3·75	3·63	3·35	4·75	3·74
180	4·35	3·85	4·35	3·85	4·75	4·23
210	4·35	3·85	5·35	4·95	4·90	4·88

Table XII and Fig. 4 summarize the results for the absorption and Table XIII and Fig. 4 for the excretion of water. On Fig. 5 the rates of urine formation in the rat, rabbit and guinea-pig are compared.

DISCUSSION.

The object of this paper has been to study the normal relationship between alimentary absorption, tissue hydration and renal excretion of water in the rabbit, guinea-pig and rat. In the guinea-pig and rat the rate of water absorption was studied statistically by killing sets of animals at varying times after water administration. But in the rabbit, owing to great variability in the weight of the gut and its contents, this method was found to be unsuitable. In rabbits therefore the absorption of water was studied indirectly by observing changes in the water content of small muscle samples taken with the aid of local anæsthesia. Our controls confirm the observations of Tashiro [1926] and Baer [1927] that the water uptake of different muscles in the same animal may be unequal, so that from changes in the water content of any single muscle it is not possible to draw accurate quantitative conclusions as to the total increase in water content of the body musculature. In our experiments no appreciable increase in the water content of symmetrical parts of the longissimus dorsi muscles was observed during the progress of a normal water diuresis in rabbits. If, however, the diuresis is interrupted by pituitrin so that the absorbed water is no longer excreted an increase is regularly observed in the water content of this muscle, and if this degree of increase were representative of the entire musculature, a large proportion of the administered water would be accounted for. So we may conclude that by our method it is possible to detect any appreciable storage of water in muscle.

Since there is no regular appreciable increase in the water content of rabbit muscle during a normal water diuresis it is very probable indeed

that no great storage of water takes place unless the excretion of urine is lessened (as by pituitrin). The average increase of muscle water of twelve animals was 0·33 p.c., and this in a 2 kg. animal with, say, 950 g. of muscle would represent less than 13 c.c. of additional water.

The results obtained on skin samples are more variable, but they are liable to greater errors. Sometimes larger increases in their water content are observed than have been described for the samples of muscle, but since the average weight of the skin in nine rabbits amounts to only 12 p.c. of body weight these increases do not represent more than a few c.c. of additional water.

In guinea-pigs and in rats it is clear, however, that water absorption is well in advance of diuresis. The direct study of water absorption from the gut is more difficult and less accurate in guinea-pigs than in rats, since the gut forms as much as 20 p.c. of the total body weight in the former and 5 or 6 p.c. in the latter animal. When our results on guinea-pigs are treated statistically it is probable, however, that most (4/5ths) of a 5 p.c. body weight dose of water is absorbed in 60 min. and practically certain that the maximum load of absorbed but unexcreted water occurs some 20–40 min. before the height of the diuresis. In rats this is still more clearly demonstrated because of the greater certainty of our results on water absorption.

There is no doubt that in rats, and it is highly probable in guinea-pigs, that the maximum load of water in the blood and tissues precedes the period of maximum diuresis, and that there may be no increase in urinary output at a time when a quantity of water equivalent to 3 litres in man has been absorbed. A statistical survey of the water content of muscle and liver from rats which had and rats which had not received water, demonstrates clearly the additional water content of these tissues at a time when there is no appreciable diuresis (see Tables X and XI, and Fig. 3). The water increase in liver is rather greater than in muscle, perhaps due to the greater water content of portal blood during absorption. The delay in the onset of diuresis has been often attributed to the fact that the portal blood must first pass through the liver. Although the liver takes up proportionally more water than muscle it is certain from our results that in the rat the total amount of water held in the liver is insufficient to cause any appreciable delay in diuresis. Thus when 5 p.c. body weight of water is given the amount of water held in the liver is less than 0·5 p.c. body weight even with abnormally large livers. This does not mean that the liver cannot be responsible for the delay in diuresis. Indeed Mollitor and Pick report a shortening of this delay

in dogs with an Eck fistula. It would appear, however, that it cannot be due to delay in the entrance of the water into the general circulation as a result of its storage in the liver, since the degree of storage is insufficient.

It would appear to be demonstrated quite clearly that the rate of urine formation at any moment is not proportional to the amount of extra water then present in the blood and tissues. There is a time lag of 20 or more min. between the maximum water load and the maximum diuresis.

In Fig. 2 it will also be observed that in rats the water-load curve falls below the base line. The urinary excretion and extrarenal excretion of water is therefore carried beyond the need of the animal to dispose of its excess. The total urinary output after giving 5 p.c. body weight of water averaged only 3·35 p.c. body weight in 3 hours and the non-fæcal extrarenal water loss was 3·69 p.c. body weight in the same time. The large extrarenal water loss was the main factor in carrying the water-load curve below the base line, since at this time the urinary output had lessened. Nevertheless it will be shown in Part II that the fall of the water-load curve does not determine this difference in magnitude between the dose of water given and the output of urine.

Observations made by Priestley upon human subjects have indicated that following the administration of large doses of water the general blood dilution, as estimated by hæmoglobin and the dried weight of blood, is slight, but that a dilution of electrolytes is readily demonstrated. Priestley attributes these changes to the withdrawal of salts from the blood: a natural consequence of the storage of water in tissues. In the rat this storage of water is demonstrated both by direct analysis of tissues and by the simultaneous study of water absorption, urinary output and the loss of water extrarenally. In the guinea-pig water storage also takes place.

In the rabbit, however, it has been shown in this paper that there is comparatively little water storage and that water excretion appears to lag only a little behind water absorption. One would, therefore, anticipate that in the rabbit the reduction in the electrolytes of the blood would not be so marked. This has been shown to be the case by one of us [Smirk, 1932].

Further evidence upon this is offered in a subsequent paper of this series. Verney explains the delay in the onset of diuresis by the time required for the disappearance of the pituitary hormone from the blood. Such a delay might arise in this way or from the time required to mobilize

some diuretic substance from the tissues. It is not likely that a delay of as much as 20–30 min. would be observed if water diuresis were the result of a direct action of the nervous system upon the kidney.

Conclusions.

1. In the guinea-pig and in the rat alimentary absorption is well in advance of diuresis. In rats, given 5 p.c. of their body weight of water, a weight equivalent to 3 litres of water for a man is usually absorbed at a time when no increase in urinary output has taken place.

2. The rate of uptake of water from the gut of a rabbit is probably not much more rapid than the rate of excretion. There are, however, difficulties in the quantitative interpretation of results upon which this observation is based.

3. The rate of urine formation is not proportional to the then existing degree of hydration in muscle, liver and blood.

We desire to thank Profs. T. R. Elliott and E. P. Pick for their helpful criticism and advice, and Dr Priestley for his kindness in affording us an opportunity for discussion.

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Part II. The influence of binding an animal, of its body temperature, and of extrarenal losses of water upon diuresis.

Introduction.

The influence of the binding of a rabbit upon its urinary output is intimately related to changes in body temperature and extrarenal losses of water. It would be difficult to consider these subjects separately.

Thus when an unanæsthetized rabbit is bound lightly on its back its temperature falls. If it is bound and kept in a room at body temperature then its temperature rises; and the increase in temperature of bound rabbits in a room at 37° C. is greater than the relatively slight increase in temperature of the unbound animals.

In preliminary experiments we found that bound rabbits kept in rooms at ordinary temperature had a normal urinary output: but in rooms at 37° C. a marked inhibition of urinary output. Unbound animals in rooms at 37° C. had slighter urinary inhibition. The degree to which such reduced outputs of urine could be attributed to increased extrarenal losses of water was next investigated and an attempt made to separate experimentally the effects of temperature and of extrarenal loss upon diuresis.

The effect of lightly binding an unanæsthetized rabbit upon its temperature and diuresis after water.

It has been stated by Molitor [1926] that as a result of the fright induced in an unanæsthetized rabbit by the simple act of binding it to an operating table an inhibition of diuresis results, the mechanism of which is a central nervous system reflex independent of the posture in which the animal is held. On the other hand, it is also well known that an increase in the urinary output may co-exist with emotional over-anxiety in man.

In our preliminary controls however we did not observe any reduction in the urinary output of bound animals. But since any such alterations might prove a fruitful source of misinterpretation it was thought advisable to pursue the matter further.

Results.

In a series of ten rabbits the diuresis which resulted from a given dose of water was compared with the diuresis from an equal dose of water subsequently administered to the same animals under similar conditions,

adding the one additional procedure of lightly binding the animal on its back to a small operating table. It was found that the average urinary outputs of bound and unbound animals did not differ appreciably (Table I), but that the rectal temperature of the bound animals had fallen—the average fall being 1·7° C.

TABLE I. The influence of lightly binding an animal upon its body temperature and upon diuresis.

	Unbound		Bound and allowed to cool	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.
1	106	—	88	−1·6
2	57	—	86	−2·6
3	87	—	89	−1·9
4	75	0·0	105	−1·1
5	69	−0·2	80	−1·5
6	67	−0·1	64	−1·9
7	106	−0·3	79	−1·5
8	126	—	135	—
9	109	—	57	—
10	42	—	57	—
Average	84·4	−0·2	84·0	−1·7

These experiments differ only from those described by Molitor in that we allowed the body temperature of the bound animals to fall whereas Molitor took steps to prevent cooling.

It would appear that the simple act of binding together with the resulting fall of body temperature does not produce any significant change in water output.

An inhibition of diuresis co-exists with a rise in body temperature.

Molitor prevented his bound animals from cooling by the use of heated tables, electric lamps or warm air chambers at 37° C., but he does not quote the body temperatures produced by these measures.

The temperature of our rabbits in a chamber at 37° C. rose, and the diuresis after giving water was considerably reduced not only in the bound animals but also in the unbound controls. The temperature increase was greater in the bound animals as was also the reduction in urinary output.

The average urinary output of the seven bound animals kept in a warm chamber was 16 c.c. and their average temperature increase 2·4° C., whereas under normal conditions their output was 81 c.c. and they had an average fall in temperature of 0·2° C. (Table III).

The average output of the five unbound animals in the warm air chamber was 37·5 c.c. and their average temperature increase was only 0·3° C. Under normal conditions these same rabbits had an output of 75·5 c.c. and a fall in temperature of 0·2° C. (Table II).

TABLE II. The effect of a warm environment upon an unbound animal.

	Unbound and not warmed		Unbound and warmed	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.
1	106	Average of 12 other animals −0·2	42	+0·2
2	57		30	+0·0
3	87	—	39	+0·6
8	61	−0·5	39	+0·0
9	66	−0·2	38	+0·8
Average	75·5	−0·2	37·5	+0·3

TABLE III. The effect of a warm environment upon an animal gently bound on its back.

	Unbound and not warmed		Bound and warmed	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.
1	106	Average of 12 other animals −0·2	29	+1·8
2	57		16	+2·8
3	87		11	+2·4
4	75		24	+3·0
5	69		18	+3·7
6	67	−0·1	6	+2·1
7	106	−0·3	9	+1·2
Average	81	−0·2	16	+2·4

It would therefore appear that a rise in the temperature of a rabbit produced by keeping the surrounding air at body temperature is regularly associated with a very considerable lessening of diuresis.

Does the simple act of binding cause an inhibition of diuresis if the fall of body temperature produced by binding is prevented?

On a series of eleven rabbits the diuresis produced by 75 c.c. of water was compared in the same animals when bound and unbound. The rectal temperature was measured frequently during the experiment and in bound animals the temperature was maintained, where possible a fraction of a degree below the initial temperature, by transferring the animal to a warm chamber whenever the body temperature showed a tendency to fall.

In some animals the temperature fell more than was desirable. Group I, Table IV, comprises the animals where the temperature has not fallen more than 0·5° C. below the temperature in the control experiment, and Group II, Table V, comprises those animals where the temperature has fallen by more than this amount although as a rule less than in the animals of Table I where no attempt was made to prevent cooling.

TABLES IV and V. The effect of temperature changes upon the diuresis of rabbits.

TABLE IV. Group I.

	Normal		Bound and temperature maintained normal	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.
10	51	−0·3	15	−0·7
12	34	−0·2	50	−0·6
14	30	−0·2	17	−0·7
17	60	−0·5	20	−1·0
18	59 (cabbage)	−0·2	69	−0·4
19	101	−0·4	80	−0·6
20	35	−0·3	41	−0·5
Average	53	−0·3	42	−0·6

TABLE V. Group II.

	Normal		Bound and temperature slightly reduced	
Animal No.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.	Diuresis c.c. in 4 hr.	Maximum temperature change in ° C.
11	20	−0·1	93	−0·9
13	29	−0·3	67	−1·7
15	43	−0·0	37	−1·4
16	64	−0·2	59	−1·9
Average	39	−0·15	64	−1·5

It will be seen from Table IV that the total urine formed by the seven animals of Group I was 42 c.c. in 4 hours when bound and the temperature was kept normal as against 53 c.c. when they were unbound. In Group II, which contains four animals, the fall in temperature averaged 1·5° C., and the total output when the animals were bound amounted to 64 c.c. as against 39 c.c. when they were unbound.

It would appear therefore that the act of binding may have some influence in inhibiting diuresis, since in experiments performed on the same series of animals maintained at the same level of body temperature and subjected to the same manipulations of bladder expression and measurements of rectal temperature, the additional procedure of binding

seemingly reduced the urinary output in four out of seven animals, the reduction in urinary output being 31 p.c.

It must be remembered, however, that when bound the animals had their rectal temperatures measured more frequently, and were being moved between a warm and perhaps stuffy chamber and a cool room according to the level of their rectal temperatures.

For the experiments described in Tables IV and V only animals were used with a fairly constant urinary output in successive diuresis tests.

It is clearly very difficult to separate the effect of binding from the changes in body temperature even when these are slight. It is possible that the direction in which the mechanisms of heat regulation are acting (hot → cold or cold → hot) play a part before any significant temperature change is observed.

The relationship of extrarenal loss of water to diuresis in rabbits.

A series of five rabbits previously fed on cabbage and oats and allowed an unlimited supply of water were deprived of food and water overnight. The diuresis and non-fæcal extrarenal losses were then determined over a period of 4 hours, after giving 4 p.c. of their body weights of warm water by stomach tube. The results of this experiment, conducted at ordinary room temperature (17° C.), were compared with the results obtained when a similar experiment was carried out in a room warmed to 35–36° C. In all experiments the animals were unbound.

It will be clear from Table VI that the sum of the average urinary and extrarenal losses of water is approximately equal in the two series

TABLE VI. The relation between renal and extrarenal water losses at different temperatures.

	Control observation at 17° C.		Experiment at 35–36° C.		
Experiment No.	Extrarenal loss g. in 3 hr.	Diuresis c.c. in 3 hr.	Extrarenal loss g. in 3 hr.	Diuresis c.c. in 3 hr.	Maximum temperature increase in °C.
1	13·5	30·5	51·0	26·0	+2·4
2	15·0	93·0	30·0	74·0	+0·9
3	10·0	93·0	41·5	38·5	+1·7
4	24·0	56·0	48·0	24·0	+1·6
5	24·5	46·5	88·5	22·5	+1·6
Average	17·4	63·8	51·8	37·0	+1·6

of results, but whereas in the experiments conducted at laboratory temperature the urinary output of water exceeds the extrarenal loss, the reverse is the case in the warm chamber. There is in fact a clear inhibition

of diuresis which would appear to be sufficiently accounted for by the increase in extrarenal loss of water.

It will be observed, however, that in experiments conducted at 35–36° C. there is a distinct increase of body temperature, and although

TABLE VII. The effect of previous water depletion upon a water diuresis.

	Controls (no previous water depletion)			Experiments			
Rabbit No.	Water given by stomach tube = 4 p.c. body weight c.c.	Amount of water in excess of normal c.c.	Diuresis during the subsequent 4 hr. c.c.	Amount of previous water depletion c.c.	Water given by stomach tube c.c.	Amount of water in excess of normal c.c.	Diuresis during the subsequent 4 hr. c.c.
22	88	88	78	70	88	18	61
23	91	91	86	85	91	6	43
24	81	81	71	67	81	14	65
25	90	90	38	67	90	23	99
26	86	86	17	59	86	27	71
27	123	123	129	44	123	79	107
28	112	112	91	57	112	55	64
29	114	114	62	49	114	65	70
30	105	105	127	43	105	62	86
31	114	114	114	46	114	68	60
Average	100·4	100·4	81·3	58·7	100·4	41·7	72·6

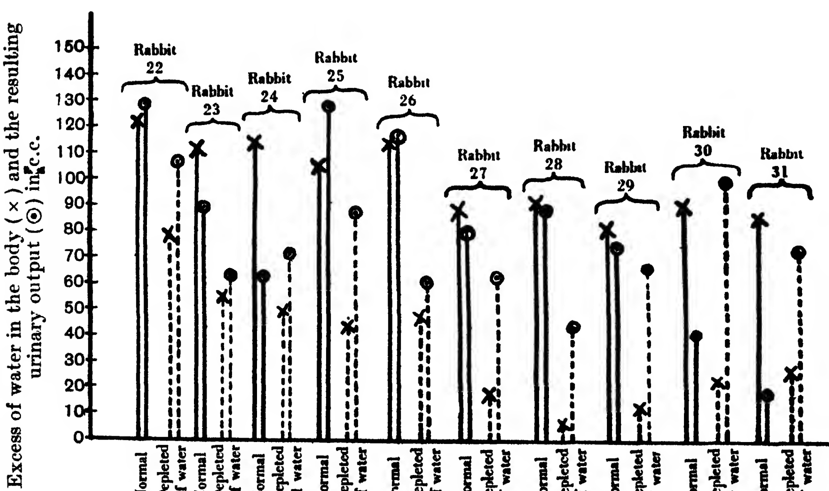

Fig. 1. The effect of previous water depletion upon a water diuresis.

extrarenal losses of water are without doubt increased it does not follow that the inhibition of urinary output is a result of this loss. It was decided therefore to produce a corresponding degree of extrarenal water

loss in a series of rabbits by keeping them in a warm chamber at 36° C., allow them to cool to normal body temperature and then to administer water. In this way it should be possible to separate the effects of extrarenal loss from those due to the slight increase of temperature.

It will be seen from Table VII and from Fig. 1 that although a previous water depletion usually diminishes the diuresis from a subsequent dose of water, the degree of diminution when present does not correspond to the amount of water lost and is always less than this.

The amount of water given by stomach tube was the same both in the experiments and in the controls, and averaged 100·4 c.c. in each. In the controls where there was no water depletion this amount given constitutes an excess of water above the initial quantity present, but in the experiments, owing to the previous water depletion, the excess of water only averages 41·7 c.c., although the same quantity of extra water has been administered per os. The diuresis which results averages in the controls 81·3 and 72·6 c.c. in the experiments. A diminution of 58·7 c.c. in the excess of water produced by the preceding water depletion causes a fall of only 8·7 c.c. in the urinary output. In the controls the urinary output over a period of 4 hours is usually rather less than the excess of water resulting from water administration, but in the experiments the urinary output is in all cases greater than the excess of water, and is usually considerably greater.

The changes described occur so regularly in each pair of observations that it is quite fair to consider these average figures as representative.

In contrast, the urinary output obtained when the animals were in the warm chamber was less than the diuresis in the experiments which we have just described, in spite of the fact that the extrarenal loss of water had been much greater in the experiments at room temperature.

It seems clear therefore that the inhibition of diuresis in a warm environment is not mainly the result of the increased extrarenal loss of water induced.

The relationship of extrarenal loss of water to diuresis in rats.

Since it is difficult to determine the rate of water absorption from the gut of a rabbit it was thought desirable to repeat our observations on rats and to ascertain whether the diminished urinary output resulted from interference with water absorption. It is also of interest to study the relationship of temperature, extrarenal loss and diuresis in a small animal where one would expect the part played by extrarenal loss to be greater.

The water-absorption rate of rats in a room at 37° C.

A series of five rats were fed for 3 days on bread and milk. They were allowed water up to within 1 hour of the experiment, but were deprived of food for 12 hours except that by mistake they had access to dry biscuit for a short time several hours before the experiment—there was no appreciable residue in the alimentary canal at the conclusion of the experiment.

The rats were placed in the warm chamber for 1 hour and then given 5 p.c. of their body weight of warm water and returned to the chamber. At the end of 40 min. they were killed and the alimentary canals removed and weighed together with their contents and expressed as a percentage of the total body weight.

The results obtained were 8·1, 6·6, 7·7, 6·7, 6·1 p.c., averaging 7·0 p.c. From similar experiments conducted at room temperature and described in Part I [Heller and Smirk, 1932] we may conclude from the average water-absorption curve obtained (Part I, Fig. 1) that the normal average is 6·8 for animals allowed water up to the time of the experiment. It is therefore clear that there has been no appreciable delay in water absorption. Any clear inhibition of urinary output in similarly treated animals is not to be attributed to diminished alimentary absorption.

The influence of a warm environment on renal and extrarenal water elimination.

A series of eleven rats, fed on bread and milk to within 12 hours of the experiment, were weighed and placed in a large incubator at 37° C. for 1 hour. They were then re-weighed, adding to their weights the weight of any fæces passed during this hour. Their urine was collected on cotton-wool swabs and was also weighed. 5 p.c. of their original body weights of warm water was next administered and the animals returned to the incubator. The resulting urine was collected at frequent intervals and the non-fæcal extrarenal loss was again ascertained by weighing. The urinary outputs and extrarenal losses are expressed as a percentage of the animal's total body weight and are compared with the results of similar experiments upon the same animals conducted in a normal environment at ordinary room temperature.

Table VIII compares the total outputs of urine and the extrarenal losses of these animals at laboratory temperature and at 37° C. Fig. 2 gives representative diuresis curves for the animals in the different temperature environments.

TABLE VIII. The relationship between diuresis and extrarenal loss in experiments on rats. Conducted at 37° C.

Rat No.	Urinary output per 3 hr. as percentage of body weight	Extrarenal loss per 3 hr. as percentage of body weight
24	1·19	4·15
25	1·54	4·60
5	0·66	5·40
7	3·25	4·85
8	4·00	3·70
9	3·65	3·35
12	3·26	5·85
13	0·98	10·40
14	1·36	8·10
15	1·40	4·35
16	2·05	7·40
Average	2·12	5·67

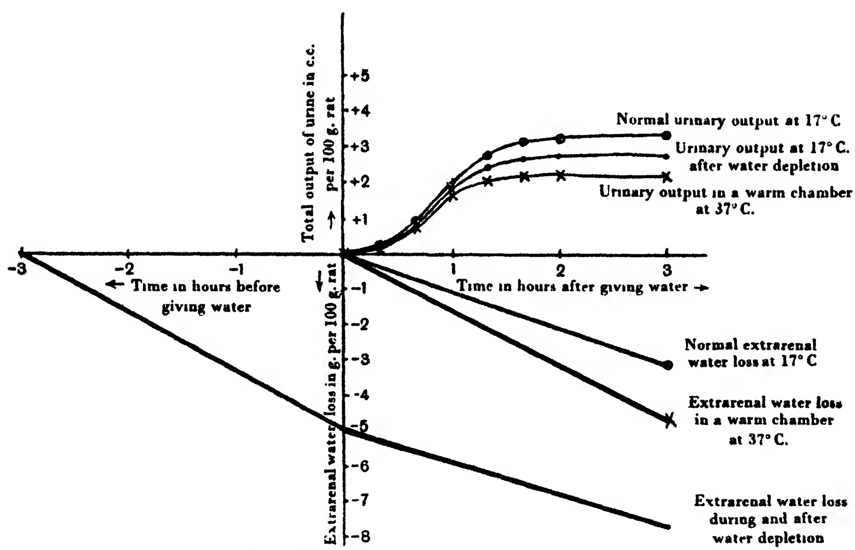

Fig. 2. The relations between environmental temperature, extrarenal water losses and diuresis.

It will be observed that there is a moderate diminution in the total urinary output and an increase in the extrarenal water elimination, the results being similar to those obtained in rabbits except for a lesser degree of renal inhibition.

Three more rats were only placed in the incubator after giving water. Their urinary outputs were 2·34, 2·92, 1·46 c.c. per 100 g. rat, and the extrarenal losses of water 6·0, 5·9 and 5·4 g. per 100 g. rat for the 3 hours.

The inhibition of diuresis is not well marked, and it appears necessary to have the animal in the warm environment for a short time before giving the water.

In five of the experiments from Table VIII the rats were removed from the warm chamber at the end of 2 hours. At this time it appeared that the secretion of urine had ceased, and from the previous experiments it seems justifiable to conclude that if they had been left in the warm chamber for the full 3 hours there would have been no further increase in urine formation during the last hour.

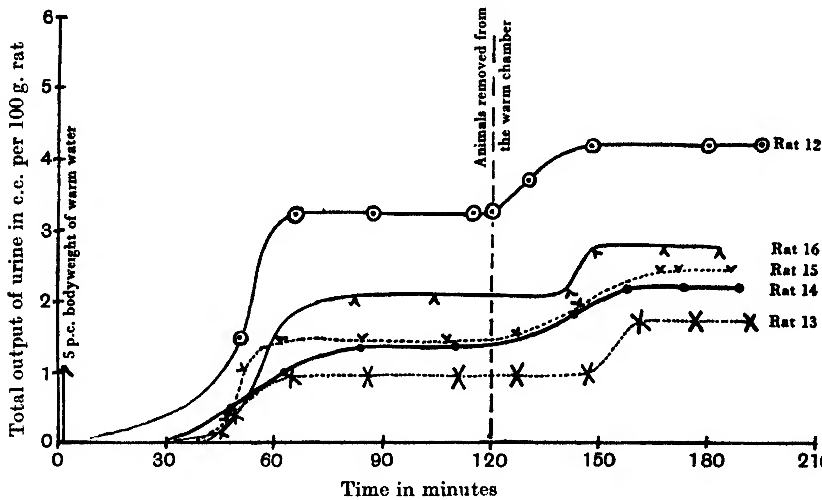

Fig. 3. Inhibition of diuresis by warmth and renewal by cold.

But although none of the animals left in the warm chamber had any appreciable increase in urine after the first 75 min. all animals removed from the warm chamber had, after an initial delay, a further increase in the urinary output (see Fig. 3).

This observation provides strong evidence that extrarenal loss of water does not sufficiently explain the urinary inhibition observed in the warm environment. If the sole explanation of the lessened urinary output was the increased extrarenal water elimination, then from the time the extrarenal water loss had been sufficiently great to inhibit urine formation one would expect no further increase in the urine secreted until the animal was given a fresh supply of water. This, however, is clearly not the case, and one must postulate some factor other than the water content of the tissues.

It is possible that a slight increase in body temperature may be present in rats as in rabbits, and may be the primary or a secondary cause of the reduced urinary output.

Water diuresis without water excess.

It is natural to suppose that water given to an animal depleted of water would first be used to make good the deficit, and that only the unneeded excess would be excreted. In the rat, as in the rabbit, it can be shown that this is not the case with moderate losses of water.

A series of eleven rats were placed in the warm chamber at 37° C. (with short intervals at room temperature) for a period of 3 hours and the non-fæcal loss of weight recorded. In most instances the loss amounted to more than 5 p.c. of the animals' weight, the normal loss at laboratory temperature being about 3·7 p.c. for a corresponding time (Table X).

The animals were then allowed about 1 hour at laboratory temperature and were given 5 p.c. of their original body weight of warm water by stomach tube. The resulting urinary outputs and extrarenal losses were determined in the manner already described, and the results again expressed as a percentage of the original body weight.

It will be seen in Table IX and Fig. 4 that the total urinary outputs are only slightly less than the outputs of the animals under normal conditions (Table X and Fig. 4), and it is quite clear from this that water diuresis follows approximately its normal course in the rat as in the rabbit when there is no excess of water present in the body, and even when there is actually a deficit of water in spite of the additional fluid administered.

TABLE IX. Diuresis without water excess in rats.

Rat No.	Extrarenal loss of water 3 hr previous to giving water by stomach tube as p.c. of body weight	Amount of water given per os as p.c. of body weight	Residual water deficit	Urinary output in the 3 hr. after giving water as p.c. of body weight	Extrarenal loss in the 3 hr. after giving water as p.c. of body weight
3	4·1	5	−0·9	3·40	2·50
4	5·1	5	0·1	1·82	2·85
5	4·1	5	−0·9	1·60	3·20
6	5·5	5	0·5	2·67	—
22	6·0	5	1·0	2·87	—
23	6·6	5	1·6	3·05	—
7	6·0	5	1·0	3·10	2·80
8	4·3	5	−0·7	3·85	3·75
9	3·9	5	−1·1	3·75	3·75
10	4·2	5	−0·8	1·71	6·30
11	5·1	5	0·1	2·52	3·3
Average	5·0	5	0·0	2·76	3·56

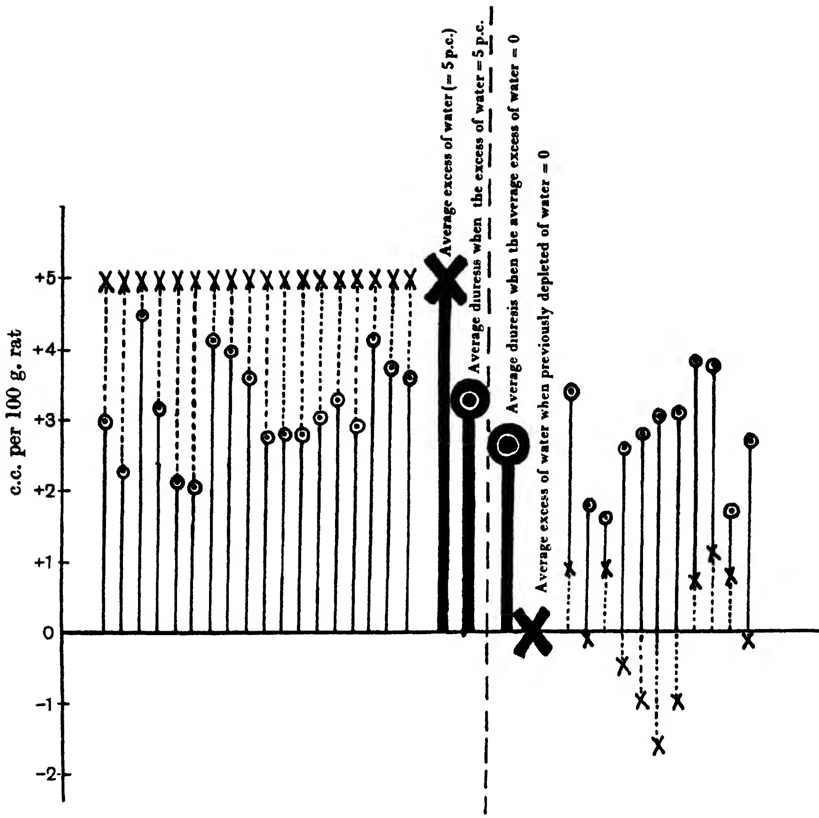

Fig. 4. Water diuresis without excessive water in the body.

Each vertical line represents one experiment. The height of the × above or below the zero line represents the excess or deficit of water in the body after the whole of the 5 p.c. body weight of water given has been absorbed, deficits being the result of previous water depletion. The height of the ⊙ above the zero line represents the urinary outputs resulting from water administration. The thick lines in the middle of the diagram represent the average values of the two groups of results.

A. Controls. (5 p.c. of body weight of water by stomach tube.)

B. Experiments. (5 p.c. of body weight of water by stomach tube, the rats being previously depleted of water [average depletion = 5 p.c.].)

TABLE X. Diuresis with water excess in rats (no previous water depletion).

Rat No.	Amount of water given per os as percentage of body weight = Excess of water in the body	Urinary output in the 3 hr. after giving water as percentage of body weight	Extrarenal loss in the 3 hr. after giving water as percentage of body weight
1	5	3·00	2·00
2	5	2·35	2·90
3	5	4·50	1·65
4	5	3·25	1·80
5	5	2·15	2·90
6	5	2·05	2·75
7	5	4·15	5·20
8	5	4·00	6·70
9	5	3·60	5·60
10	5	2·75	7·20
11	5	2·80	8·40
12	5	2·80	3·50
13	5	3·05	1·95
14	5	3·30	3·55
15	5	2·90	2·10
16	5	4·15	3·20
17	5	3·75	2·65
18	5	3·60	2·45
Average	5	3·29	3·69

It is therefore a reasonable deduction that the inhibitions of diuresis which were observed in these animals in the warm chamber could not be due to the relatively slight extrarenal losses, and in all probability were the result of the slight increases in body temperature which were observed in unbound rabbits under similar conditions (see Part I of this paper).

DISCUSSION

In small animals such as the rat and rabbit the extrarenal loss of water is large, and in the rat it frequently equals the urinary output. This loss would appear to be mainly from the respiratory tract, and since oxygen requirements are proportional to surface rather than to weight the pulmonary ventilation of small animals must be large relative to their weight. For this reason, and because the body temperature in rats is about 2° C. higher than in man, a relatively large loss of water from the lungs is to be expected.

Thus in rats the extrarenal water loss at room temperature has averaged 1·23 g. per hour per 100 g. rat, and in rabbits it has averaged 0·29 g. per hour per 100 g. rabbit. In a single experiment on a man the extrarenal loss was roughly 0·04 g. per hour per 100 g. man.

When rats or rabbits are placed in a chamber at 37° C. the necessity for heat regulation causes an increase in the respiration rate, and as one

would anticipate an increased extrarenal loss of water. At the same time the urinary output is diminished and in rabbits there is a slight increase in body temperature. (A slight increase in body temperature was also observed in the few rats in which it was measured.) It would be a reasonable supposition that the loss of water by another channel explains the lessened output of urine; but in Part I it was shown that the rate of urine formation at any given moment was not parallel to the excess of water in the body at the time. We therefore decided to explore the possibility of some other factor being the cause of the reduced urinary output.

With this object in view a series of experiments were performed in which the animals were allowed to lose water extrarenally before giving water by stomach tube. Now although the total extrarenal loss of water was greater than normal in the rabbits and rats where the diuresis was conducted in a warm chamber (Tables VI and VIII), yet it was even greater in the above-mentioned animals which were allowed to lose water extrarenally and were removed from the warm chamber some time before receiving their dose of water. Despite the greater loss of water in these latter animals their outputs of urine were considerably larger than the outputs of the rabbits which were kept in the warm chamber while diuresis was in progress.

These results are more clearly demonstrated in the rat, in which animal the extrarenal loss is relatively great. Thus, in several experiments when, despite the addition of water per os, there is still a deficit of water in the body, a typical diuresis is obtained not appreciably less than that of normal controls.

It appeared, therefore, that it was some effect of a warm environment other than increased extrarenal loss of water that was responsible for the inhibition of renal activity. It has also been shown that this factor is not the failure of water absorption. Again, 2 hours after giving water when the urinary inhibition produced by the warm chamber appeared complete, five of the rats were transferred to a cage at ordinary laboratory temperature. A second though smaller diuresis was obtained in all rats after their removal from the warm chamber although no more water had been given. But no such diuresis was obtained in the third hour in any rat left in the warm chamber.

It is clear that if extrarenal loss alone had been the factor responsible for urinary inhibition then there would have been no second diuresis in the rats removed from the warm chamber at the end of 2 hours.

Since it has been shown that the temperature of the rabbits is increased

in the warm chamber it seems not unlikely that the increase in temperature or some factor related to it is responsible for the urinary inhibitions. In favour of this may be quoted the effect of immobilizing rabbits. We have shown in the earlier part of the paper that the temperature regulation of rabbits appears to be upset if the animal is immobilized and that on placing immobilized rabbits in the warm chamber their temperatures rise by 2 or 3° C., whereas unbound animals have increases of less than 1° C. Likewise at ordinary room temperatures the body temperature is maintained in unbound rabbits but falls a degree or so in bound rabbits.

The ordinary inhibitions produced by a warm chamber at 37° C. are much more complete in bound animals where the rise of body temperature is appreciable than in the unbound animals where the rise of body temperature is slight. The balance of evidence is rather in favour of changes in body temperature being responsible for urinary inhibition.

The fact that a normal diuresis can be obtained when there is no excess of water present in the body indicates clearly that water diuresis is not a delicate expression of the body's water needs, and that it may take place when the animal's water reserves are already depleted and cause a still further reduction in the water reserves.

Part I provides a further illustration of the absence of parallelism between the water content of the tissues and urinary output at the time, and a previous paper by one of us [Smirk, 1932] confirms the observations of Priestley that diuresis is not dependent upon the increases in the water content of blood as measured by the hæmoglobin percentage.

Water diuresis does not appear therefore to depend upon an excess of water in the blood or tissues and may actually take place when the water reserves are depleted.

Conclusions.

1. In rats and rabbits a previous reduction of the water reserves of the body fails to produce an equivalent diminution in the diuresis which follows the administration of water per os.
2. In rats a typical water diuresis has been obtained when there is no excess of water in the body.
3. In air at 37° C. rabbits and rats have a reduced urinary output and an increase in their extrarenal loss of water. The increase in the extrarenal loss of water is not mainly responsible for the inhibition of renal activity. The inhibition may be caused by the slight increases in body temperature observed under these conditions.

4. The simple binding of a rabbit alters its temperature regulation to a degree which necessitates the control of this factor in diuresis experiments.

We wish to thank Prof. T. R. Elliott for his helpful criticism and advice.

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THE RELATION OF ATHLETIC STATUS TO THE PULSE RATE, IN MEN AND WOMEN.

By F. S. COTTON.

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Physiological literature contains a number of references to the fact that exceptionally slow pulse rates have been recorded in the case of highly trained athletes. Yet a search for precise data on the subject reveals the fact that very few observations have been made on it, and as far as the writer has been able to ascertain no data have been published giving the exact status of a series of athletes and their respective pulse rates.

The chief references to this topic appearing in the literature are given in the following brief review:

In 1909 Miss Buchanan, in writing "On the physiological significance of the pulse rate," thought it noteworthy to record the fact that a famous Oxford stroke had a resting pulse rate of 45. In the same year Michell published an account of observations on a large number of undergraduates at Cambridge who took up athletics during their University careers. He found that the average pulse rates (including early morning and late evening pulse rates) which were counted under nearly basal conditions were: first year 69, second year 64, and third year 56. When school athletes were excluded from the group the averages were: first year 74, second year 68, and third year 58.

Although these figures show conclusively that athletic training decreases the resting heart rate, the extent of the slowing may be exaggerated in these figures for a reason that is discussed at the conclusion of this review.

Michell [1909] further records that when a picked team of men, some of whom at least have been taking part in some form of exercise for more than 3 years (for example a University eight), is examined, the early morning frequency may be 52; the rate in some individuals being 48 or 46.

Dawson [1919], who observed the effect of physical training on pulse rate and blood-pressure during training, found that the resting

pulse rate was decreased after the period of training, but quotes no actual figures. In 1926 he further investigated the effect of athletic exercise on the resting pulse rate, this time in girls from 13 to 19 years of age.

In one set of observations the athletic girls showed an average pulse rate (recumbent) some 11 beats (85–74) below the non-athletic group, while in another set, the decrease in the athletic girls averaged 4 beats less per minute (84–80).

In 1927 Henderson, Haggard and Dolley, writing on the significance of rapid and slow pulse rates, state that "athletes frequently have very slow pulses, their rates are ten to twenty or even thirty beats slower than in men of sedentary habit." They give no group results, as these observations formed part of a wider investigation. In the protocols of other data however, the minimum resting pulse rate was 45 per minute.

In 1927 Schneider, Clark and Ring investigated the influence of physical training on the basal pulse rate in the case of five subjects who took up strenuous exercise for periods ranging from 4 to 16 weeks. The extent of the work was not less than 1 hour daily and included tennis, running, handball and swimming. The basal pulse rates were on an average slower after the period of training in the case of four out of the five subjects, to the extent of some 2 to 3 beats per minute.

Although the results are consistent with the previously recorded observations, the conclusions are vitiated by the fact that no account was taken of the possibility that a seasonal influence might be operating to produce a change in the basal pulse rate. That this might be an important factor to consider in observations extending over relatively short periods, is evident from the work of Griffiths, Pucher and others [1929], who found in four subjects a seasonal change in the basal pulse rate over a period of 2 years, with a summer minimum averaging some 7 beats per minute lower than the winter rate.

In 1929 Hoogewerf made an extensive series of electro-cardiographic observations on over 200 Olympic athletes. He mentions incidentally with regard to the pulse rate that the mean value was 50. No attempt was made, however, to grade or classify the athletes in any way, nor was any measure of variability indicated by standard deviation, probable error, etc. This aspect of the data was not developed, probably because no pulse rates were actually counted for the full minute, but were determined from the short period of the electro-cardiographic tracing.

It is therefore probable that Hoogewerf recognized that the record time was too short to yield accurate individual data, suitable for statis-

tical treatment, but that the mean pulse rate of all would approximate to the true value.

Contemporaneously with Hoogewerf's observations, Bramwell and Ellis [1929] conducted a clinical investigation on the same set of athletes. These workers were apparently more interested in other measurements, and although the resting pulse rates are given for the various groups of athletes, no special attempt was made to achieve really good conditions of rest.

Since, however, the athletes were all observed under approximately the same conditions it is of interest to pick out the runners from the tables and place them in a series together as follows:

Type of runner	Resting pulse rate
Sprinters	66
Middle distance runners	63
Long distance runners	61
Marathon runners	58

It will be seen that we have arranged the runners in order of the increasing duration of their athletic exercises. It is at least striking that the mean pulse rate decreases in each case in the same direction. Although the number in each group (15–30) is not large enough for inferences statistically sound, this relationship becomes more significant in view of the facts already reviewed, and of the data presented later in this paper.

Of all the data quoted in the foregoing, that recorded by Dawson [1926] on girls and Michell on men undergraduates are the only cases in which really systematic observations were made to assess the effect of physical training on the pulse rate.

Dawson's results appear quite convincing; but on account of the age and sex of the subjects (young girls), the amount of athletic training was probably but moderate, so that nothing exceptional was recorded in regard to really slow heart rates.

The same cannot be said with regard to Michell's data, for it is quite possible that part of the decrease in pulse rate in the three successive yearly tests may be due, not to real changes in the individuals, but to the experimental conditions operating in two ways, viz.:

(1) To an increasing degree of care on the part of the observer in ensuring complete rest of the subject. This is suggested by the figure 74 quoted as the mean pulse rate in the first-year series. This average is exceptionally high for conditions that include both early morning and late evening counts, even for an unselected set of men, whereas Michell's

group included a good many athletes. Judging from our own figures and those quoted in the literature one would not expect a higher mean pulse rate than about 66 under these conditions. It is therefore scarcely possible that the subjects were taken in the first year under adequate conditions of repose.

(2) To the possibility that increasing familiarity of the subject with the observer and the conditions of the experiment may make for some improvement in the resting state.

This criticism may be supported by the fact that the basal pulse rates in the case of three separate groups of medical students, taken under the same conditions at the University of Sydney, were lower in a second series of observations on the basal pulse rate made 12 months after the first. In the second set of observations the extent of the decrease in the pulse rate was 4, 1 and 2 beats per minute respectively in the three groups.

It is worth noting in passing that according to Sutliff and Holt [1925], the age gradient in pulse decrease is insignificant at this time of life, so that this factor need not be considered in Michell's results nor in the second point of criticism of them.

It would seem, therefore, that the objections advanced above could only be removed by obtaining the basal pulse rates of a group of subjects, and dividing them into sub-groups on the basis of extent of athletic training, and by reducing the effect of individual variation to a negligible amount by making the numbers in the sub-groups sufficiently large. This was attempted in a set of observations which are described in section 2 of this paper.

The same procedure appears already to have been adopted by Pembrey and his co-workers in analysing the pulse data of some 94 men and 103 women.

As the writer has not been able to obtain the original reference to discover how the division into athletic and non-athletic subjects was made, he can make no comment on the data other than draw attention to the fact that greater numbers than those observed would be necessary to stabilize the difference in pulse rate between the two constituent groups of subjects.

A summary of Pembrey's [1922] results is given by Lamb [1930] in his book on Human Physiology. The mean value for trained subjects was 61 as against 72 for the untrained subjects in the case of men, and for the corresponding groups of trained and untrained women 75 and 84 respectively.

The objects of the present communication are two in number:

(1) To present the basal pulse rate data in the case of two highly trained groups of athletes (one of which shows the lowest mean basal pulse rate yet published), and to record in this connection the exact status of each athlete on a quantitative basis.

(2) To describe an investigation of the variation in basal pulse rate in both men and women according to extent of physical training.

SECTION 1.

Dealing with quantitative athletic status and basal pulse rate of exceptional athletes.

It is naturally a difficult matter to obtain extensive data along these lines, for not only is the field of subjects highly restricted, but it is no easy matter to realize the conditions necessary for determining their basal pulse rates.

In the year 1921, however, an opportunity arose for obtaining these data, when a number of selected athletes, including Olympic competitors from abroad, were grouped together at the same hotel while taking part in the swimming championships of Australia. The pulse rates were counted after a night's rest, the subjects remaining recumbent in bed after waking, until the observations had been made. Several counts were made on successive mornings on each subject.

The essential data are given in Table I, together with a detailed account of the status of each athlete.

It should be noted that the group is small, since it is restricted to those having to their credit at least a championship of one of the states of Australia. In addition each athlete has at least been placed first or second in an Australian swimming championship, and two of them have each had the distinction of being the world's best middle distance swimmer of his day.

The quantitative status of each athlete is assessed by expressing his best performance (officially timed in a championship race), as a percentage of the existing world's best for the distance. For example, let T_s be the subject's best official time for a given distance and let T_w be the world's record for the same. Then, since the quality of a performance varies inversely as the time, the quantitative status is given by

$$\frac{T_w}{T_s} \times 100.$$

Ten years later, in 1931, a further opportunity presented itself for the recording of data from another group of athletes of exceptional calibre.

Table I. Basal pulse rates and status of a number of champion swimmers.

Initials	General status of athlete (Ol. A.) = Olympic Athlete	Quantitative status. $\frac{\text{World's record}}{\text{Athlete's best}} \times 100$	Basal pulse rate: Mean	Basal pulse rate: Minimum	Weight in kg. stripped	Age in years	No. of years of strenuous athletic training
L. L.	Holder of world's record for $\frac{1}{2}$ mile (Ol. A.)	100·0	52	47	70·0	30	14
F. E. B.	Winner of innumerable championships from 220 yd. to 1 mile in Australia and Europe over a period of 15 years (Ol. A.)	99·9	50	49	65·9	30	15
P. K.	Won several Australian championships. Placed second 100 metres, Olympic Games, 1920; representative of U.S.A. (Ol. A.)	98·1	42	41	80·9	19	3
F. S. C.	Winner of $\frac{1}{4}$ mile and $\frac{1}{2}$ mile championships of N.S.W. (Australia) and second in $\frac{1}{4}$ mile championship of Australia	95·6	40	38	65·5	31	12
I. S.	Won many state championships of Victoria (Australia) over a period of years. Established Australian record for 220 yd. breast stroke (Ol. A.)	94·8	53	52	72·7	30	12
M. C.	Placed second in $\frac{3}{4}$ mile championship of Australia. Subsequently won many Australian championships from $\frac{1}{4}$ to 1 mile (Ol. A.)	94·7	47	41	65·9	21	4
S. S.	Winner of several championships of Queensland (Australia) from $\frac{1}{4}$ to 1 mile	93·8	49	46	56·4	31	13
S. B.	Winner of breast-stroke and back-stroke championships of N.S.W. Winner of 220 yd. breast-stroke championship of Australia	91·3	47	43	59·1	24	5
	Mean	96·0	47·5	44·6	67·0	27	10

Table II. Basal pulse rates and status of highly trained middle distance runners.

Initials	General status of athlete	Quantitative status. $\frac{\text{World's record}}{\text{Athlete's best}} \times 100$	Basal pulse rate: Mean	Basal pulse rate: Minimum	Weight in kg. stripped	Age in years	No. of years of strenuous athletic training
J. H. B.	Winner $\frac{1}{2}$ mile championship, Sydney University, 1930. Winner $\frac{1}{4}$ mile open scratch race N.S W. (Australia)	94·8	50	48	81·8	22	5
P. H. W.	Winner mile championship, Sydney University, 1929, 1930, 1931. Ex-holder University mile record	93·8	62	60	61·8	21	5
K. S. J.	Holder of N.S.W. junior state record for $\frac{1}{2}$ mile	93·1	58	56	66·0	20	4
R. B. T.	Winner $\frac{1}{2}$ mile championship, Sydney University, 1931. Holder University 1 and 2 miles record	92·2	49	48	64·5	20	4
G. R. J.	Half-mile runner. No championships to his credit	91·2	53	50	73·1	18	3
	Mean	93·0	54·4	52·4	69·4	20	4

Although only five in number these men form far the best and most highly trained group of middle distance runners ever present at the University of Sydney. The data, set out in the same way as in Table I, are given in Table II.

Comments on Tables I and II.

It is a striking fact that Bramwell and Ellis found the lowest pulse rates of all groups of Olympic athletes examined to occur in the case of the Marathon runners, where the mean age was greater than in the other group. They say "the Marathon runners are quite a distinct type. They are much older men, in the late twenties and early thirties." The mean age given for the group was 27 years. This is suggestive that the effect of exercise in decreasing the heart rate continues to operate steadily over a period of years. It will be noted that the mean age of the group of swimmers of Table I is also unusually high—27 years—five of the eight being over 30 years of age. Moreover, four of these five were then at the apex of their physical condition, inasmuch as during that year they recorded better performances than ever before.

The number of years of hard physical training is on that account much greater (10 years) than in Michell's rowing eight (3–4 years), or of the group of half-mile runners of Table II (4 years).

In explanation of the long periods of training quoted in certain cases in Table I, it should be stressed that these are quite conservative. It may be mentioned that it is not uncommon for champion swimmers to mature early[1] and to maintain their form for much longer periods than most athletes. No better example can be given than in the case of F. E. B. of Table I who at the age of 14½ years won the quarter-mile championship of Victoria, and who won in all 32 championships of Australia commencing at the age of 15 and concluding his series at the age of 33. In addition his first and last appearance as a representative of Australia and place winner at the Olympic Games, were spaced 16 years apart (1908 and 1924).

Whether or no these are the influences that have operated to produce the lowest mean basal pulse rate for a group yet recorded, it is not possible to say, as the number is too small for statistical significance. Only an accumulation of such data could settle the question.

[1] For example, a world's record has been broken by a swimmer of 15 years of age, a thing without parallel in any other sport. The same swimmer (A. Charlton of Australia) at the age of 16 won the 1500 metres race in 20 min. 6⅗ sec. at the Olympic Games (Paris, 1924), again breaking the world's record for the distance.

Section 2.

Dealing with quantitative athletic status and basal pulse rate of athletes of varied calibre.

The object of the observations now to be described was to obtain data as to the effect of athletic exercise on the pulse rate in such a way as to obviate any fallacies such as may have affected the observations recorded by Michell.

In presenting the results it is felt that still larger numbers of subjects in the groups would have been desirable, but as the data were necessarily obtained in different years and not always under the same conditions of rest or time of day for different groups, these have been kept separate. On the other hand, the fact that all groups show the same kind of change, though varying in degree, reduces to the vanishing point the possibility that the differences occur by chance.

The method of grading any one group of subjects (whose individual pulse rates were all determined under the same conditions) into various sub-groups according to their athletic history was in the first instance as follows:

A general questionnaire was prepared setting out in tabular form all the common forms of sport, recreation and athletic exercises. The subjects, who were practically all medical students (between the ages of 19 and 21 years), were required to fill in an account of their activities in each section, and to indicate the degree of proficiency attained. For example, a subject might record that he had played first grade football for two seasons, had run a mile in a certain time, and so on.

Upon examination of the forms an attempt was made to classify the subjects into four groups:

(1) Those of less athletic calibre than the average.
(2) Those of average athletic calibre.
(3) Those of rather higher athletic calibre than the average.
(4) Those of a distinctly high athletic calibre.

Although a somewhat different set of groupings might have resulted if the completed forms had been sorted by different investigators, the writer was satisfied that a reasonably good sub-division had been arrived at when all had been considered.

Certain guiding principles were employed in the assessment of the athletic histories, *e.g.* a first grade footballer, playing for several seasons, would be placed in the fourth group, whereas a second grade footballer would be placed in the third group. Again, the fact that a subject had

run the mile in something of the order of 5 min. would qualify him for group 4, while a performance of 5½ min. for the mile would qualify for the third group. Further, where several sports or games had to be considered collectively, football and rowing were regarded as more strenuous than hockey, the latter more strenuous than cricket, and so on. In the case of swimming, the specified quotation of certain times for distances was a valuable guide.

In all there were 121 subjects in this first investigation, who were grouped as described.

As the number in the fourth group was too small, it was decided to amalgamate this with group 3, and thus to compare the basal pulse rates for the three main subdivisions:

(1) Those of less athletic calibre.
(2) Those of average athletic calibre.
(3) Those of greater athletic calibre.

The results were rather unexpected, as very little difference was shown in the three groups, viz.:

Group 1.	Mean basal pulse rate	61·9
Group 2.	,,	61·7
Group 3.	,,	59·5

The writer found it difficult to understand why so small a difference as 2·5 beats per minute should appear between the extreme groups, in view of the profound decrease in the pulse rate of the group of athletes in Table I. It seemed possible that a distinct effect in lowering the pulse rate might only be produced by very intensive physical training, and that in the group examined there were too few heavily trained subjects to make much impression upon the group averages. Further, in the case of the two groups given in Tables I and II, their calibre is fixed by definitely measurable criteria which lends significance to the results in spite of paucity of numbers, whereas in the student groups errors of classification may operate to quite an unknown extent.

As the results were not encouraging the observations were abandoned, and were only revived many years later upon the consideration of a fresh point of view. In 1930 it was decided to re-investigate the matter by changing the method of grouping.

Now it often happens that a first grade footballer or a first class runner may owe his pre-eminence more to innate physical ability[1] than

[1] This disturbing factor would be negligible where athletes of the calibre of those in Tables I and II are concerned, since in these cases competition is so keen as to demand hard training on the part of the athlete.

to actual training, and may have undergone less actual physical exercise than, say, a second grade footballer or a second class runner. Wherever this occurs the grouping would run counter to the principle of sorting aimed at, and the result of such occurrences would tend to "blur" the distinctness of the groups.

On the other hand, practically every individual indulging in various games and sports comes into contact with a good many of his peers, and has every opportunity of gauging the relative extent of his own training in relation to the rest.

In consideration of this fact it was decided to attempt a fresh mode of grouping where the classification was made by the individual himself in relation to his fellows. Instead then of an elaborate questionnaire each subject was asked to consider carefully the totality of physical exercise of every description that he had ever undertaken, and to classify himself into one of the following groups:

(1) A group having had a history of less athletic exercise than the average.

(2) A group having had a history of about an average amount of athletic exercise.

(3) A group having had a history of more athletic exercise than the average.

Here again some overlapping must inevitably occur owing to absence of measurable criteria, so that any real difference would thereby be diminished. In spite of this, the first group examined in this way showed a distinct difference in the sub-groups, as did each subsequent group without exception, even when the numbers were relatively small. This was true not only of the results of the basal pulse rate determinations, but also when observations were made under daytime conditions, both

(*a*) when all the subjects of a group were rested sitting for a period of not less than 1 hour, and

(*b*) when all the subjects were taken sitting but for a short period of rest only.

The results for each group are given separately in Table III.

It should be emphasized that each individual pulse rate from which the sub-group average was determined, was itself a mean of not less than six counts on the subject, and for the most part on anything from three to six separate occasions.

It is clear, therefore, that each individual value was a reliable one, and on this account any tendency of chance variations to blur the distinctness of the group mean would be minimized.

TABLE III. Difference in the mean pulse rates of groups of subjects (males) classified according to the extent of their athletic history.

Conditions under which pulse rates were taken	No. of group	Sub-groups: Less athletic history	Average athletic history	Greater athletic history	Column 3 minus column 5	Mean for whole group	No. of subjects in group
Basal	1	64·7	58·9	54·6	10·1	58·4	72
	2	65·3	63·0	57·7	7·6	62·3	42
	3	66·1	63·1	56·0	10·1	61·7	37
	4	67·3	63·0	60·9	6·4	62·8	30
Mean or total	—	—	—	—	8·8*	62·2†	181
Daytime	5	74·1	72·6	65·5	8·6	70·4	50
	6	79·1	76·4	71·5	7·6	74·6	60
	7	82·5	70·0	65·1	17·4	70·5	84
	8	80·1	78·0	73·9	6·2	77·4	43
Mean or total	—	—	—	—	10·9*	71·7†	237

* It should be noted that the means of the differences in pulse rate are not simply the means of the numbers appearing in the column labelled (column 3 minus column 5), but the weighted mean of the numbers, thus allowing the larger groups to exercise their due influence on the final mean.

† In obtaining the means for all groups in the basal condition, group 1 is omitted, as it was not strictly comparable with the others, for the reason explained subsequently in the text. Group 8 is omitted from the daytime average for the same reason. Here the true resting condition was not aimed at, and the group results are therefore distinctly elevated.

Comments on Table III.

The relatively low rates of group 1 are to be explained by the elimination of the higher counts of the series of individual basal pulse rates in obtaining a mean individual figure. Every subject shows a certain tendency to vary, even on the same occasion as well as at different times under apparently the same conditions. By elimination of the higher values and retaining the lower consistent figures, the results approximate nearer to "true basal" pulse rates as distinct from "mean basal" pulse rates, which are given as the average of all determinations under the same standard basal conditions.

The remaining groups 2, 3 and 4, on the other hand, show mean basal pulse rates closely consistent with those published in the literature. Here the average of all groups is 62·2, whereas the following values are given by various writers: Korosy [1911] 63·3, Harris and Benedict [1919] 61·3, and Sutliff and Holt [1925] from personal observations and from a thorough search of the literature, 62 to the nearest whole number.

Although the means of the corresponding sub-groups are not amalgamated, owing to variation of conditions, the extreme sub-group differences (given under column 3 minus column 5) are averaged, since any variation in conditions of a whole group affected equally both these sub-groups.

It will be seen that the average difference under daytime conditions (10·9) is greater than the corresponding difference under basal conditions (8·8). This might, however, be expected, as the daytime pulse rate is itself distinctly higher than the basal pulse rate. When the extreme sub-group difference is expressed as a percentage of the mean for the corresponding whole group, this discrepancy largely disappears. Thus the extreme sub-group difference of 8·8 under basal conditions equals 14 p.c. of the corresponding mean for the whole series, while in the daytime series the mean figure 10·9 equals 15 p.c. of the corresponding general mean.

It was found possible to obtain but one series of observations in the case of women. In this group the resting sitting pulse rates were determined after a short period of rest (some 15–20 min.), in most instances after walking a few hundred yards. The subjects were practically all between 17 and 19 years of age. The results are given in Table IV.

TABLE IV. Effect of athletic history on the daytime sitting pulse rate in the case of women.

Less athletic history	Average athletic history	Greater athletic history	Column 2 minus column 3	Mean pulse rate	No. of subjects
90·1	84·2	82·2	7·9	84·5	80

The mean pulse rate shows the same relation to increased athletic exercise as in the case of the men, namely, a distinctly slower rate in the more athletic group.

The mean value, moreover, is approximately the same as the figure quoted in the literature for women sitting at rest, viz. 84.

For the purpose of ready comparison the various sets of data for basal pulse rates in the present section are collected together in Table V.

TABLE V. Mean basal pulse rates of men, grouped in ascending order of extent of athletic training.

Class of men	Authority quoted	Mean basal pulse rate
Less athletic history	This paper	66
Average athletic history	This paper	63
Greater athletic history	This paper and Michell	57
Superior University blues	This paper and Michell's rowing eight	53
Olympic athletes	Hoogewerf	50
Group of swimmers averaging ten years' athletic training (mostly superior Olympic athletes)	This paper	47

Making due allowance for the fact that some of the groups are few in number, it is striking how the basal pulse rate invariably decreases in the table with increasing intensity and duration of athletic training.

SUMMARY.

1. A brief review is given, drawing attention to the prevailing agreement that slow pulse rates are characteristic of highly trained athletes, and to the need for an attempt to develop the quantitative aspect of the subject.

2. Details of basal pulse rates, together with a quantitative estimate of athletic status, are given for a group of eight highly trained swimmers (each at least a state champion). The mean basal pulse rate was 47, which is the lowest group value yet recorded.

3. An attempt to trace the relation between the slowing of the pulse rate with extent of athletic history in the case of 121 men as gauged by a questionnaire failed to yield convincing results.

4. A similar attempt showed marked differences between sub-groups of 237 men, when the basis of grading was made upon the subject's estimate of his quantitative athletic history, in comparison with his fellows.

5. Confirmatory results were obtained in the case of the resting daytime pulse rates of 80 women, who were asked to grade themselves in the same way.

In conclusion I wish to express my thanks to Prof. H. W. Davies, from whose laboratory this communication is published, for his encouragement and criticism, and to all those who acted as subjects for observation.

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[1] Quoted from Lamb.

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"POSTURAL REVERSAL" IN PERIPHERAL PREPARATIONS.

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"Postural reversal" has been defined as "the determination of the type of response to a given stimulus by the limb posture existing when the stimulus is applied" [Forbes, 1922]. Magnus [1910] showed that a cat's tail can be drawn reflexly from either side towards the median plane when stimulated at the tip, the direction of the movement being determined by the initial passive posture of the tail. The reaction might, with equal aptness, be termed "movement reversal." Magnus showed that the shunting effect could be traced to the influence of the deep afferents, and was therefore tonic. Sherrington [1900] found in reflex preparations that certain stimuli would cause flexion if the limb were already passively extended and extension if it were flexed.

The object of the present paper is to show that "postural reversal" can be demonstrated in peripheral preparations with direct stimulation of motor nerves, and to suggest that as well as a central factor in the grading and co-ordination of muscular movements there is a peripheral factor, namely, the changes in receptivity caused in the muscles by varying degrees of stretch.

This investigation began some years ago in an experimental study of the muscular movements of the diaphragm [Briscoe, 1920]. Tracings were taken simultaneously from the crus and the dome, and the stretch of the two parts relatively to each other was altered by flexing or extending the spine. Extending the spine stretches the crus, flexing stretches the dome. Both in movements due to natural respiratory impulses, and in those due to electrical stimuli, it was noticed that the contraction started earlier in the crus when the spine was extended and earlier in the dome when the spine was flexed. The extent of contraction was also shown to be

related to the degree of stretch, both with natural and artificial stimuli. In a pithed animal, the phrenic was stimulated by single break-shocks of varying (but near threshold) strength. In these experiments the "threshold" stimulus was taken to be that strength of stimulus which just produced a recordable movement in the muscle. It was found that, with a just minimal stimulus, the crus did not shorten if the back was flexed, and the dome did not shorten if the back was extended. In other words, the visible response to a threshold stimulus per the phrenic nerve was found to be dependent on the posture of the body causing different degrees of stretch in the two parts of the diaphragm. This change of threshold for movement was later demonstrated in the gastrocnemius of cats and rabbits, the muscle being left in its natural relationships and stretched or slackened by fixing the foot in different positions [Briscoe, 1924]. Break-shocks near threshold value were used, and it was found that the reaction was obtained with much greater regularity when the nerve was not cut off from the central nervous system. The suggestion was made, therefore, that the threshold reaction to stretch was dependent upon the integrity of the reflex arc. Ordinary single shocks of threshold value do not produce regular minimal contractions in a peripheral preparation, but in the present paper it is shown that the visible threshold reaction to stretch can be demonstrated with great regularity, even when the nerve has been severed, if the stimuli employed resemble natural impulses more closely than the single break-shock.

METHODS.

The experiments have been performed on cats, under ether and chloralose or dial anæsthesia. The double nerve muscle preparation used has been usually the tibialis-gastrocnemius pair, the muscles being left undisturbed and their contractions recorded by the movements of the foot. Sometimes the gastrocnemius-soleus combination has been pitted against tibialis and extensor longus digitorum. When necessary the movements of the individual tendons have also been recorded.

The method of stimulation employed has been that described in a previous article [Briscoe and Leyshon, 1929], namely, the regular intermittent discharges of a neon lamp. The discharges are led through the primary coil of an induction apparatus. Each nerve with its stimulating pair of electrodes is connected to one of two secondary coils, which are then placed on either side of the primary coil. The presence of a second coil makes no difference to the stimuli provided by the first coil. By use

of a key in the primary circuit, simultaneous excitation in the secondary circuits can be ensured.

Results.

If muscles are kept in their natural relationships they can be stretched or slackened by moving the bony points to which they are attached. Thus the flexor and extensor groups working the ankle joint can be stretched and slackened alternately by performing passive movements on the foot, the leg and thigh being fixed by drills. These passive changes in length are of considerable importance. The preparation is so arranged that the thigh is vertical and the leg is horizontal. No particular posture is imposed on the foot, which is free to be moved either by passive traction, or by the contractions of the muscles working over the ankle joint. The foot usually takes up a neutral position, neither flexed nor extended, at an angle of about 50 degrees or 60 degrees to the horizontal, and if alternate passive movements of flexion and extension are carried out, the foot will return to approximately the same neutral position. If, however, the foot is carried passively into a position of extreme flexion and held there for a few seconds, on release the limb will not return to the neutral position, but will remain in a position of semi-flexion. If this procedure is repeated in the opposite direction the foot, on release, will take up a position of semi-extension.

The same effect can be obtained by making brief repeated passive movements in the desired direction, the return of the limb on release not being controlled in any way. It is found that the postures of semi-flexion and semi-extension thus imposed and passively maintained may vary by as much as 30 degrees, though 20 degrees and 15 degrees is the more usual range. In old cats the range may not be more than 10 degrees. The actual angles read depend a good deal on the way the preparation is set up; what is important is the range of passive posture which can be imposed and maintained without further effort, either external or intrinsic. That these changes in passive length are not in any way dependent on the central nervous system is readily demonstrated. Before the nerves are touched the leg and foot are fixed in position by drills and passive movements are performed or postures externally imposed, first in one direction and then in another. The sciatic nerve is then cut and the procedure repeated. Severance from the spinal centres makes no appreciable difference to the range of passive posture.

The reaction is shown in the following way. The leg is denervated by

cutting the sciatic and femoral trunks. The motor branches supplying the antagonist muscles used are dissected out, and each connected with one or other secondary coil. The foot is in the neutral position, *i.e.* neither flexed nor extended. If other postures have already been imposed the neutral position can be reassumed by making quick alternate passive

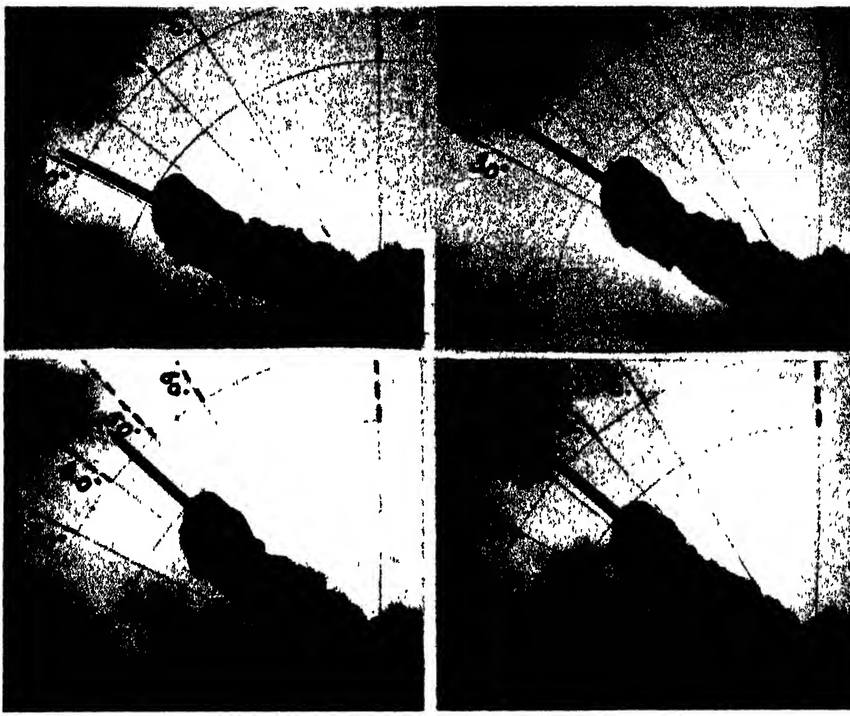

Fig. 1. The left-hand figures show the foot in the positions of passive extension (P.E.) 32°, and of passive flexion (P.F.) 46°. The right-hand figures show position of foot after the reversed movements have taken place, identical stimuli producing opposite results. Camera conditions remain constant.

movements of flexion and extension. The secondary coil used for excitation of one motor nerve, *e.g.* flexor, is placed at such a distance from the primary that a very small movement of flexion is perceptible when the circuit is closed for a brief time. This procedure is repeated on the opposing muscle using the other secondary coil. A passive posture of semi-flexion is now imposed on the foot. When both nerves are simultaneously excited, a movement of extension towards the neutral position

results. The foot is now placed in a position of passive extension. On stimulation as before, a movement of f l e x i o n takes place towards the neutral position. This is the reaction of "postural reversal," *i.e.* in each case the same stimuli are applied to the respective nerves, but the direction of movement is determined by the posture existing at the moment

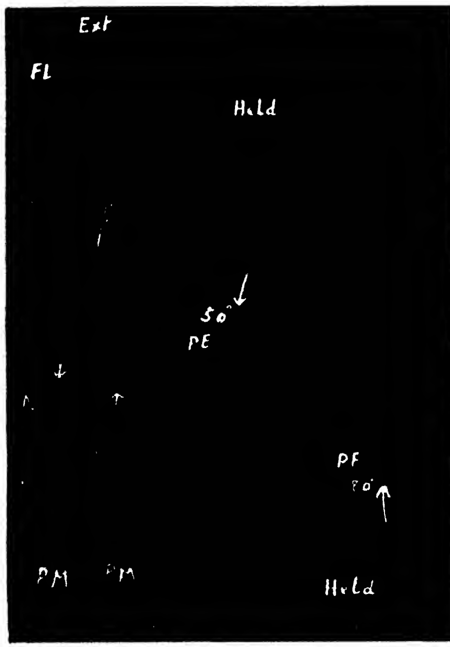

Fig. 2. Record of movement of foot. Upwards extension, downwards flexion. After alternate passive movements (P.M.) foot returns to the neutral position *N*. Stimuli to flexor and extensor (signals at top) tested separately, both give small contractions in the neutral position. Primary circuit now broken and both secondary circuits closed. After holding foot in position of full extension for a few seconds, on release it remains passively in a position of partial extension (P.E.) 50°. At the arrow both nerves stimulated simultaneously by closing the primary circuit; a movement towards flexion takes place. From partial flexion (P.F.) 80°, with the same stimuli, a movement towards extension occurs. Time in seconds.

of application (Fig. 1). If the foot is now placed in the neutral position and the same stimuli applied, usually no movement takes place, though a slight movement may occur if the muscles are not perfectly balanced. Once the right conditions have been secured, this reaction can be repeated over and over again. It is more easily obtained in preparations in which there is a large range of passive posture (Fig. 2), but it

has been demonstrated where the range of passive posture was only 10 degrees[1].

If, instead of foot movement, minimal movements of the individual tendons are recorded, the same reversal of response is seen with change of passive posture (see Fig. 3).

This change in response resulting from stretch can be demonstrated in other ways than by change of passive posture. It can be shown when stretch is produced by active postural contraction of the opposing

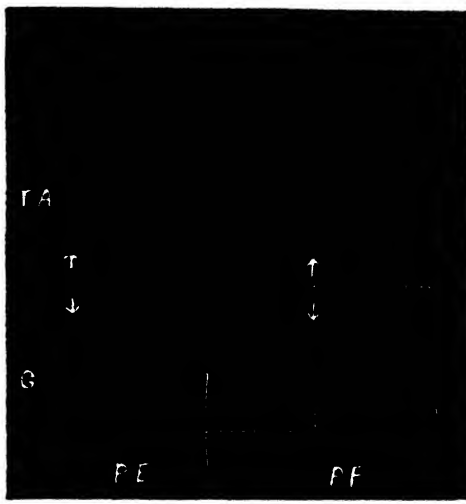

Fig. 3. Recorded from tendons. Tibialis (T.A.), median head of gastrocnemius *G*. In both cases contraction upwards. Buzzer indicates when primary circuit closed. Both secondary circuits closed. Near threshold stimuli to both nerves. When the foot is in passive extension (P.E.), tibialis only shortens and gastrocnemius is lengthened by its pull. In passive flexion (P.F.), gastrocnemius shortens and tibialis is lengthened.

muscle. This is best seen when the muscle to be stretched (gastrocnemius) is stimulated by currents which vary rhythmically in strength so that regular phasic contractions result, and the stretching muscle is stimulated by currents of constant strength causing a steady contraction. The strength of the phasic stimuli entering the extensor nerve is reduced until they are just below threshold for movement in the unstretched muscle, and there is no visible contraction. The constant stimuli entering the flexor nerve are of such a strength that the foot is drawn into a position

[1] In one pair of muscles the gastrocnemius lengthened from 121 mm. to 125 mm. in passing from a passive posture of 45 degrees to 60 degrees. The tibialis lengthened from 95 mm. to 98 mm. with the same range of passive posture.

of active flexion. This stretches the gastrocnemius, the threshold for movement is altered and the subliminal stimuli to that muscle now become supraliminal, and regular phasic extensions of the foot take place from a posture of flexion. If flexor ceases to contract and to stretch extensor, the phasic stimuli become subliminal again (Fig. 4).

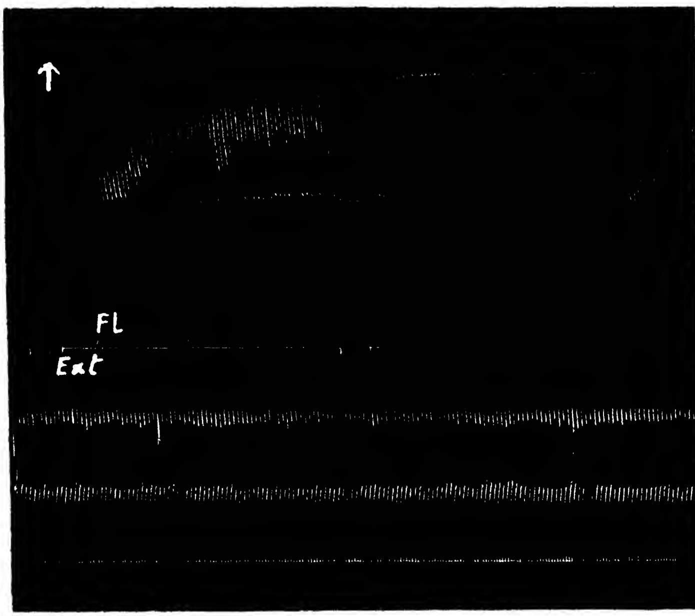

Fig. 4. Movement of foot. Flexion downwards. Primary coil stationary. Flexor secondary coil stationary—submaximal stimuli of constant strength enter flexor nerve. Extensor coil approaches and recedes from primary—phasic stimuli enter extensor nerve. Movement of coil recorded in second line from bottom. Stimuli to extensor are first shown to be subliminal ↑; when the muscle is stretched by the postural contraction of flexor they become supraliminal. The short lapse in extensor excitation shows the effect of flexor alone. When the action of flexor is removed the stimuli to extensor again become ineffective, as soon as the muscle has shortened sufficiently. Time in seconds.

The change of response is best seen in flexor when that muscle is subjected to phasic stretch. This is secured by supplying the stretching muscle (extensor) with currents which vary rhythmically in strength.

The possibility that these changes in response might be due to slight movements of the nerve on the electrodes must be considered. Control experiments were done in which the nerve was deliberately slipped over the electrodes, but care was taken to see that there was no tension or pull

on the nerve terminals. This procedure, which necessarily brings fresh pieces of nerve in contact with the electrodes, does not produce the characteristic reaction. The reaction has been obtained both with the

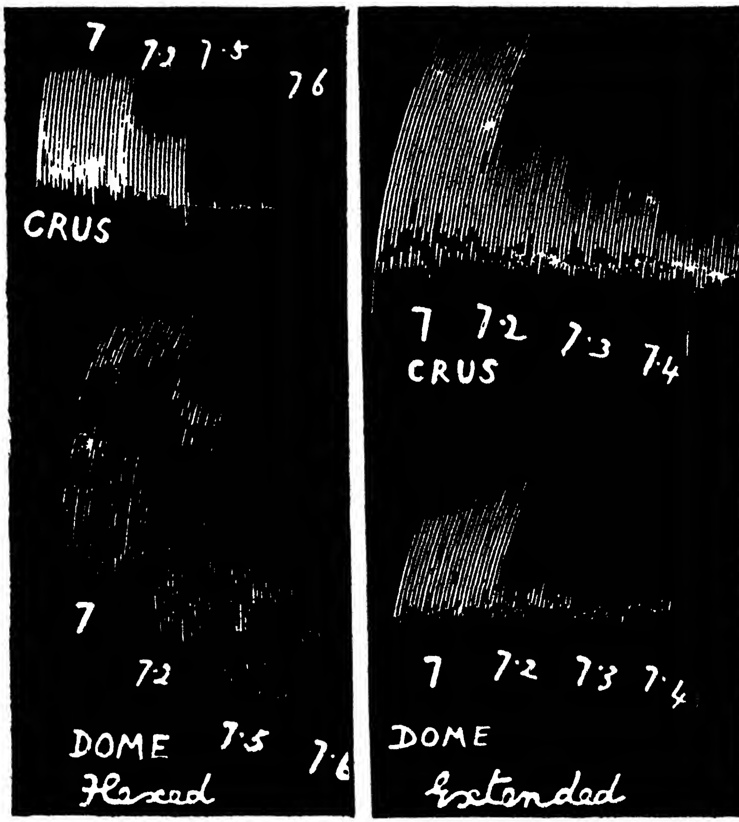

Fig. 5. Diaphragm of cat. Stimulation of cut phrenic with phasically modulated stimuli, strength reduced in small steps. Figures give distance of secondary from primary coil, coreless inductorium. In left-hand tracing spine is flexed. A near threshold stimulus (7·6) is effective in the dome and not in the crus. In right-hand tracing spine is extended. A threshold stimulus is effective in the crus and not in the dome. The sudden downward movement at 7·4 is due to a gasp of the chest muscles.

nerve lying on the electrodes without any tension and, on the other hand, with the nerve, attached by a thread to the electrode holder, under continuous tension. As a further control the phrenic diaphragm experiments have been repeated, using, instead of break induction shocks, the phasically modulated stimuli which have been shown to be capable of

reproducing accurately the deliberate movements of the diaphragm in ordinary respiration [Briscoe, 1928]. Since the nerve fibres to the crus and dome run in the same trunk, only one set of electrodes is necessary and the excitation conditions are identical, whatever the relative positions of the two parts of the muscle.

Fig. 5 illustrates this point. Records from crus and dome were obtained by means of a pneumothorax, and the muscle was paralysed by

Fig. 6. Movement of foot. Ordinates, recorded on stationary drum, show arc of lever and the limits to which foot can be carried by gentle traction. Between the angles of 75° (P.F.) and 60° (P.E.) the foot remains in any position it is placed. Arrows mark the tying of ligatures on the sciatic trunk; resultant movement is dependent on initial passive posture. In neutral position, 67°, stimulus of ligature produces small double movement, in which flexion always precedes extension. Time in seconds.

section of the phrenic nerve. Phasic stimuli of sufficient strength were used to produce movements of moderate size in both parts when the animal was lying flat. When the strength of the stimuli was reduced progressively, different results were seen according to the posture of the spine. When the back was extended, the crus was stretched, and minimal stimuli were effective in producing small movements, but were ineffective in the relaxed dome. When the trunk was flexed minimal stimuli were effective for the stretched dome, but were subliminal for the relaxed crus.

Finally, the reaction of reversal of movement can be demonstrated without the intervention of any apparatus for stimulation or record. The

only dissection necessary is exposure of the sciatic in the thigh. The foot being placed in different passive postures, ligatures tied around the sciatic trunk below the hamstring branches will produce results dependent on the initial posture. Sharply defined movements of flexion or extension will be seen if the ligatures are made with a single quick tie. As many as twenty ligatures have been applied in succession to the sciatic trunk, and in each case the direction of movement in response to the stimulus was determined by the initial passive posture of the foot. The effect of six of these ligatures is shown in Fig. 6, recorded on slow and fast rates of drum. In these experiments the stimulus is applied indiscriminately to all the fibres of the sciatic, yet clean movements of reversal can be obtained.

These control experiments all suggest that the reversal of movement is due to relative changes in the receptive conditions of the muscles concerned, and not to changes in the excitation conditions at the electrodes.

Discussion.

It is well recognized that in reflex preparations there is considerable variation in the threshold value of stimuli, so much so that this characteristic has been regarded as one of the salient points of difference between conduction in reflex arcs and conduction in peripheral nerve trunks. One of the factors producing this variability of response is the condition of passive stretch in which the reflexly contracting muscle finds itself on reception of the stimulus. Liddell and Sherrington [1924] state that "initial passive stretch often renders a seemingly subliminal stimulus submaximal, thus in appearance lowering the threshold of the reflex. A single shock that produced no visible contraction in the slightly stretched muscle will on stretching the muscle a little further evoke an obvious reflex contraction."

The experiments described in the present investigation indicate that a similar apparent lowering of threshold in response to stretch takes place even in nerve-muscle preparations. Subliminal stimuli applied to a peripheral nerve trunk become effective in causing visible contraction of the muscle it supplies when the stretch of that muscle is slightly increased. When antagonistic muscles are tested by simultaneous excitation of their nerves, the different and reciprocal degrees of stretch which ensue under varying passive postures are sufficient to bring about reversal of movement in the limb.

The regular response of an extensor muscle to a constant reflex stimulus is intensified if a flexion reflex is elicited in one of the intervals

between the stimuli [Sherrington, 1906]. Such a result can be imitated in part in a nerve-muscle preparation by causing an active or passive flexion between two extensor movements. The increase in movement which can be obtained in this way is illustrated in Fig. 7. The effect, however, is not so lasting as in a reflex preparation, as there is no after-discharge, and the augmentation ceases as soon as the muscle has returned to its shortened condition.

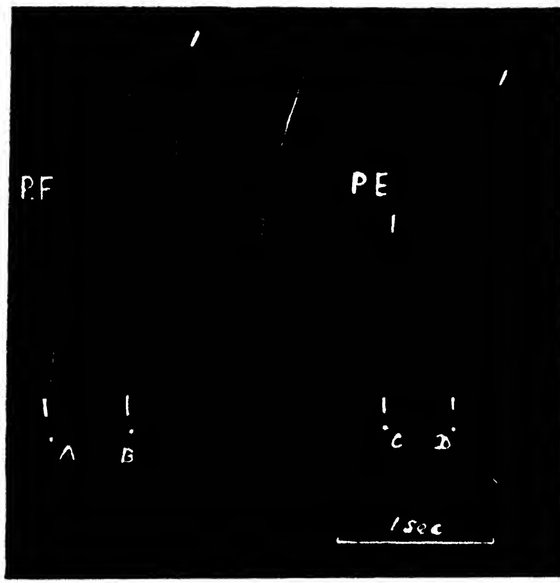

Fig. 7. Single phasic contractions of gastrocnemius, recorded by foot movement. Lower line shows movement of secondary coil, as it approaches and recedes from the primary coil. After a passive movement of flexion, the muscle has increased initial stretch (P.F.), and starts contraction when the secondary coil reaches the point *A*. After a passive movement of extension, the initial stretch is diminished (P.E.) and contraction starts when the coil reaches the point *C*, *i.e.* when the stimuli are stronger. The two relaxation curves show practically the same time relations.

The original observation that the moment of entry of crus and dome respectively in natural respiration is regulated by the posture of the spine can be repeated in the muscles of the leg. With a given modulated stimulus a movement starts earlier when the muscle is under greater stretch than when it is under less stretch (see Fig. 7).

It may be asked if this reaction to stretch is a factor in the smooth co-ordination of opposing muscles. An analysis of co-ordinated move-

ments experimentally obtained [1929] suggests that such may be the case. Records of movements of the limb caused by each muscle acting alone are necessary, as well as records of the movements under reciprocal contraction.

It can be shown that two single movements which appear unlikely to produce a smooth double movement, yet often succeed in doing so, as the moment of entry of one muscle is regulated by the stretch produced in it by its opponent (Fig. 8). This reaction helps to explain the somewhat

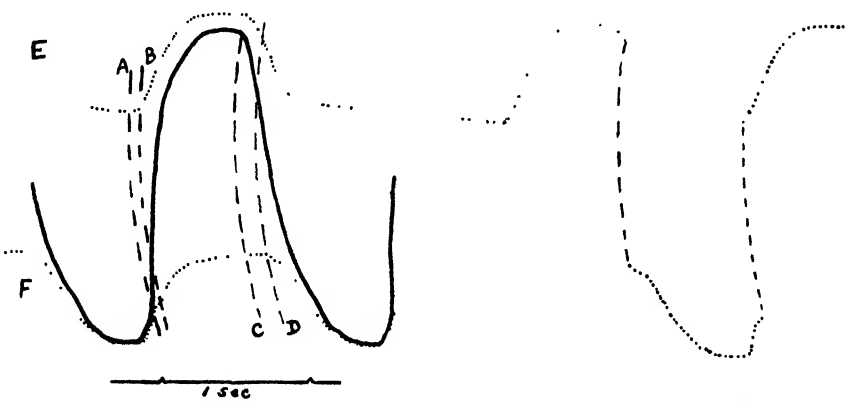

Fig. 8. Movement of foot, extension up, flexion down. Dotted lines show single separate movements of flexor *F* and extensor *E*; tracings superimposed on record of double movement (whole line) when the two muscles work together reciprocally. Time lines (broken) show arc of lever and earlier entry of muscles when stretched by their opponents. A. Start of extensor in reciprocal action. B. Start of extensor in single action. C. Start of flexor in reciprocal action. D. Start of flexor in single action. Right-hand figure shows the double movement which might have been expected from the single movements.

surprising ease with which smoothly co-ordinated movements are obtained experimentally.

The reversal of movement as seen in peripheral preparations may be due either to a balancing of smaller contractions against larger contractions, the extent of contraction in each case being governed by the amount of individual stretch, or it may be due to changes in the excitability of different muscle fibres, so that more fibres come into action with a given stimulus when stretch is increased.

It does not seem possible on the present data to decide between these two possibilities, although the observation that with a modulated stimulus a movement starts earlier in a muscle under increased stretch favours the latter alternative.

As a result of these experiments it is suggested that when a reaction of "postural" or "movement reversal" is seen in reflex preparations the mechanism is really twofold; though the central factor is responsible for the arrival of an increased number of impulses of proprioceptive origin (as the result of the stretch), yet the peripheral factor also does its part by increasing the receptivity of the muscle stretched.

Summary.

Within certain limits variations in the length of muscles in their natural relationships can be maintained passively.

These passive postures are useful in permitting study of the receptivity of muscles under different degrees of natural stretch. "Postural" or "movement reversal" can be shown in the absence of the reflex arc by making use of peripheral factors only.

It is suggested that the reversal of movement is caused by changes in the receptivity of the opposing muscles, the constant stimuli applied to the respective nerves being just subliminal for visible response when the muscles are under slight stretch and just supraliminal when they are under increased stretch.

It seems likely that this change of receptivity is a factor in the smooth co-ordination of antagonistic muscles and in the changes of reflex response to different degrees of stretch. In the intact organism this peripheral adjustment in the executant part of the reflex arc probably supplements the reinforcement provided by reflex stimuli as the result of stretch.

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RESEARCHES ON THE CONTRACTURE OF SKELETAL MUSCLE.

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I. Introduction.

The literature of contracture of skeletal muscle is extensive. Gasser [1930] has recently devoted to it a critical review. Points of importance which emerge are that (1) contractures seemingly quite diverse have yet features in common; (2) many reversible contractures in their essential chemistry resemble normal contraction. All alike show formation of lactic acid [see Gasser, 1930], splitting of phosphagen and production of ammonia. Iodoacetic acid prevents formation of lactic acid in contracture just as in contraction [Bethe, Norpoth and Huf, 1931]. The chemistry of contracture seems thus not to differ qualitatively from that of contraction.

Further, the researches of Hartree and Hill [1922] on veratrine contracture have shown quantitative similarity between the energetics of contracture and contraction. "The efficiency—measured by the ratio H/Tl—with which prolonged contraction is maintained is almost exactly the same in veratrine contraction and in normal tetanus." The same conclusion is reached by the myothermic researches of Hartree and Hill [1924, 1928], and of Furusawa and Hartree [1926] on caffein contracture and the contracture provoked by direct stimuli of greatly supramaximal intensity; also for this latter contracture by the analyses of Meyerhof and Lohmann [1925]. We seem to have before us here a broadly applicable fact.

Its theoretical importance is great. It shows that the reversible contracture of skeletal muscle is neither extreme slowing of the process of relaxation nor the operation of a special contractile apparatus, myofibrillar, or—on Bottazzi's [1897, 1926] hypothesis—sarcoplasmic.

Since reversible contractures neither in chemical process nor in mode of energy turn-over depart essentially from ordinary tetanus the differences between the two must precede the final mechanical and thermal

steps. One is led thus to approach contracture by the path of muscular excitability, especially the reversible contractures which are reactions of the muscle fibre to a stimulus, electrical or chemical.

Reversible contracture of skeletal muscle might be the result of a muscular excitation whose characteristic was slowness of development and subsidence, with absence of wave propagation. This hypothesis is borne out by the facts obtaining for direct and indirect electrical excitation of contracture, as also by the electromyogram of the "neuro-muscular" contracture of the anural Amphibia.

II. Material and general method.

The experiments have been mostly on various Anura and the cat. In the former the tendency of any given muscle, especially gastrocnemius, to respond to excitation by contracture is variable. Two factors of influence are species and nutrition. *R. temporaria* especially tends to contracture, a tendency favoured by vigorous condition of the muscle and by resistance to fatigue, therefore probably by richness in reserve nutriment. The tendency decreases under captivity.

My experiments have been on the muscles *in situ* with circulation intact (decerebrate). The contractions have been recorded, either isotonically on the kymograph with light levers (5–10 g.), or isometrically and optically with the torsion-wire type of myograph of Sherrington of rapid natural frequency, and photographed along with the electromyogram given by the string galvanometer (Cambridge new model).

III. Galvanic contracture.

If the reversible contractures of skeletal muscle connote an excitability of extreme slowness, that slowness should reveal itself (1) by high chronaxie, (2) by possibility to evoke contracture by "addition latente," *e.g.* summation of direct electrical stimuli too brief to be singly adequate, (3) also by summation of successive motor nerve impulses.

It is well known that closing or opening a galvanic circuit in series with frog muscle can excite a response which, owing to greater or less contracture, exceeds in duration the twitches excited by stimuli, indirect or direct, which are quite brief. Biedermann [1895] studied this galvanic contracture particularly in relation to the direction of the stimulating current. I have tested the influence of the duration of the electrical stimulus upon the contracture. I have excited the gastrocnemius of *R. temporaria* lengthwise by two AgCl electrodes, at tendon

and proximal pole of the muscle respectively, the contractions being registered isotonically. Under these conditions muscles tending even strongly to galvanic contracture respond by twitches free from all contracture to brief single stimuli—break shocks or feeble condenser discharges—adjusted to give contractions of comparable "height." In general the contracturing action of the galvanic current is more marked, or present only, for the "ascending" current. Fig. 1 A shows the contracture from an ascending current (cathode proximal), while a descending current of the same strength (Fig. 1 B) excites only a twitch without

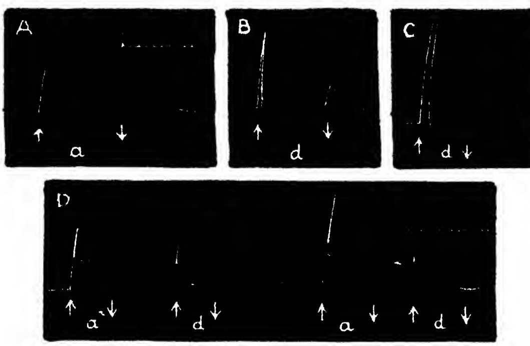


Fig. 1. Galvanic contracture of the gastrocnemius of *R. temporaria* "longitudinally" excited. Isotonic lever with a load of 5 g. Make and break of current marked by arrows pointing upwards or downwards respectively. Reduced by $\frac{1}{3}$. A. Ascending current (*a*) of 1·5 volts. B. Descending current (*d*) of 1·5 volts. C. Descending current of 2·5 volts. D. An experiment (performed on another preparation) showing the contracture producing action of the cathode and the inhibitory action of the anode. Explanation in the text.

contracture at closure, at opening a smaller twitch but clearly prolonged by contracture. When the current is ascending the greater part of the muscle is submitted to the contracturing action of the virtual cathode, near the proximal pole of the muscle; with the opposite direction of current (cathode at tendon) the main bulk of the muscle is under the inhibiting (relaxing) influence of the anode. In Fig. 1 C, with a strong descending current, the twitch at opening has quite the appearance of "post-inhibitory rebound." This relaxatory inhibition appears more clearly still in Fig. 1 D. First an ascending current is sent into the muscle and provokes a twitch followed by strong contracture which persists after cessation of the current. Some seconds later a descending current is sent into the muscle: its commencement causes a weak twitch

closely followed by relaxation of the contracture. This last recurs directly the inhibitory current stops. The figure shows a further example. This inhibitory effect of the anode on galvanic contracture proves the active character of this contracture. A similar anodal inhibition was shown by Biedermann for veratrine contracture.

In experiments on gastrocnemius of *R. temporaria* in the cold (11–14° C.) I have found it possible to separate to some extent contracture from contraction, and to measure the chronaxie of the former. Exciting

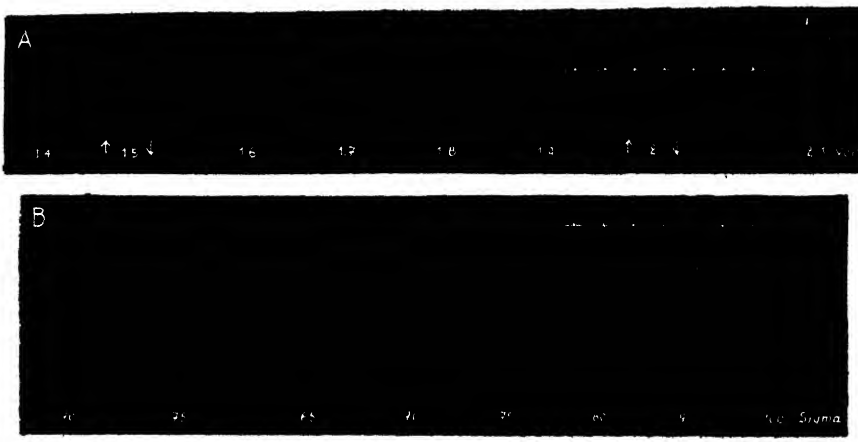


Fig. 2. Galvanic contracture of the gastrocnemius of *R. temporaria*. Isotonic lever. Load 5 g. Temp. 11·5°. Time in seconds. Reduced by ½. A. Determination of the threshold (rheobase) of contracture. B. Determination of the chronaxie of contracture. For explanation see text. Notice the diminished height of the twitches associated with contracture. This phenomenon, which is constant, is apparently explained by a very early increase in the viscosity of the muscle, which decreases its speed of shortening and diminishes the inertia of the lever.

the muscle with currents of increasing voltage, contracture can appear regularly before any trace of the ordinary contraction (Fig. 2A). This latter does not occur until considerably higher current intensities are reached. Hence one can measure the threshold for contracture and determine approximately its chronaxie by the well-known rapid method of Lapicque, namely, to double the rheobasic voltage and to find the shortest duration which the stimulus of doubled intensity must have in order to excite. Fig. 2 B gives a determination, at 11·5° C. The chronaxie of the contracture is 70–75 σ; that of the twitch contraction about 0·7 σ. The chronaxie of the contracture is about a hundredfold that of the ordinary contraction.

The galvanic contracture of mammalian skeletal muscle in the course of Wallerian degeneration of the motor nerve is absolutely similar to that of normal amphibian muscle (Fig. 3 B). The influence of duration of the stimulus is just as clear. It is seen in the different form of the twitch tension curves evoked respectively by the break and make shock of the inductorium. The make shock, which is slower than the break shock, evokes a twitch of sounder summit (Fig. 3 C). The twitches of the normal muscle reveal no trace of such a difference.

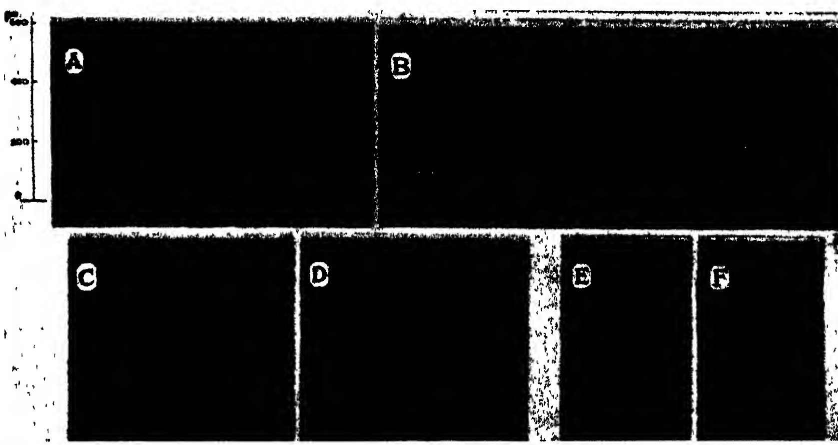


Fig. 3. Galvanic contracture of degenerated mammalian muscle (tibialis anticus of the cat, denervated 20 days previously). Longitudinal excitation. Sherrington isometric lever. Optical registration. Time in $\frac{1}{25}$ of a sec. Reduced by $\frac{1}{2}$. A. Galvanic excitation (ascending current of 2 volts) of the normal tibialis anticus. B. Similar excitation of degenerated tibialis anticus. C. Make-shock twitch, degenerated muscle (coil at 8 cm.). D. Break-shock twitch, degenerated muscle (coil at 8 cm.). E. Break-shock twitch, normal muscle (coil at 10 cm.). F. Break-shock twitch, degenerated muscle (coil at 10 cm.). Twitches in response to make shocks of the same height were identical in all respects.

This galvanic contracture of degenerating muscle is evidently the main cause of the seeming "slowness" of the galvanic twitch of the muscle under direct inspection, a "slowness" of diagnostic value to the clinician. Apart from this slow relaxation traceable to contracture, there exists certainly a slowness of the true twitch; this latter slowness attaches notably to the ascending phase of the isometric myograms given by degenerate muscles (cf. Fig. 3 A and E with B and F). This slowing of the twitch contraction of degenerate muscle is presumably related to the increase of chronaxie.

Discussion.

These experiments, confirming and extending older ones, show galvanic contracture as an outcome in the muscular fibre of a certain state of excitation capable of being inhibited by the anode. They define further the duration required for the stimulating electric current to give contracture. It is as though contracture attached to an excitable substance with a chronaxie much higher ($\times$ 100) than that of the excitable substance attaching to the ordinary "twitch"; in saying which I do not intend to imply the existence of two morphological entities coexistent in the muscle fibre to provide for two excitabilities. This approximate ratio 1 : 100 of the chronaxies of contraction and galvanic contracture in normal *temporaria* is just that found by Bourguignon [1923, 1931] for the two chronaxies of "myotonic" muscles of man, and by Kodera and Brücke [1928] for the chronaxies of contraction and contracture of initial stage of veratrinization in frog's muscle.

IV. Contracture excited by direct electrical stimuli giving "addition latente."

An electrical stimulus of given intensity though failing singly, because of its brevity, to excite contraction of a slow muscle (or, generally, the reaction, of a protoplasm of high chronaxie), can yet by repetition, of above a certain frequency or length of series, be effective, in virtue of "addition latente." This summation has been the subject of researches by Keith Lucas [1907] and of Lapicque [1925, 1926]. If galvanic contracture has for its immediate antecedent a slow excitation with a very high chronaxie, one should find "addition latente," and evoke contracture by summation of brief stimuli, for example by induction shocks applied directly to curarized muscle. Previous curarization is required of course to preclude participation by nerve.

This expectation is confirmed. Gastrocnemius (*temporaria*) was employed with its marked tendency to contracture enhanced by previous short tetanization. Complete curarization an hour beforehand, 0·001 mg. per g. frog.

Electrodes at the muscle poles, cathode proximal. Break shocks from two (or three) coils, of like construction, coreless and placed so as to avoid mutual induction. The openings of the primary circuit were adjustable to 0·1σ approximately by a Lapicque rheotome modified by replacing the closure cam by an opening cam, and adding a third opening cam. The graduation was verified by means of the string galvanometer. The stimuli were slightly supramaximal for the muscle.

Under these conditions a curarized gastrocnemius, exhibiting strong tendency to contracture with two or three break shocks given directly at variable intervals, never responds to one of the shocks given singly by other than a twitch entirely like the maximal twitch to an indirect stimulus (Fig. 4 C). So also with two similar shocks separated by an

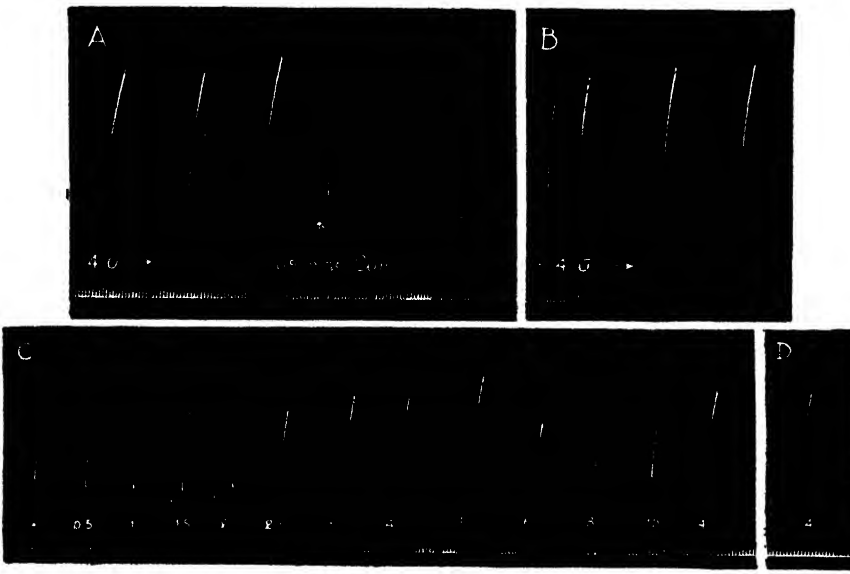


Fig. 4. Isotonic twitches of the gastrocnemius of *R. temporaria*, provoked every 2 min. Temp. 19° C. Time in seconds. Reduced by $\frac{1}{3}$. A. Twitches showing a strong neuro-muscular contracture provoked by the summation of two volleys of motor impulses at the optimum interval of 4σ. 0·05 mg. of curare was injected intravenously after the third twitch. B. Same muscle completely curarized. Contracture provoked by summation of two direct stimuli (break shocks of maximal intensity). C. Series of twitches of the same muscle showing the relation of the height of contracture with the interval between direct stimuli. D. Resistance of this same contracture to atropinization. An intravenous injection of 6 mg. of atropine sulphate between C and D.

On this figure and on the subsequent figures, single stimuli are designated by a point, and double stimuli by their interval in sigmata, under each contraction.

interval less than the absolute refractory period of the muscle measured by the least interval for tetanic summation. But, once beyond a minimal interval corresponding approximately with the least interval for tetanic summation (2σ), the isotonic twitch, now heightened because a tetanic fusion of two, is immediately followed by a secondary slow contraction (Fig. 4 B and C). The height of this secondary contraction increases at

first with increase of the interval between two stimuli up to an optimum interval of about 4σ (at 18° C.), then diminishes, first rapidly, then more and more slowly. With a greater interval than 10σ all trace of the phenomenon usually disappears (Fig. 4 C). The height of the secondary contraction can exceed that of the twitch to which it is appended. Its duration, apart from the contraction residue which prolongs it, is usually about 10 sec., but may be longer (Fig. 4 B, C, D). This secondary contraction excited by direct stimuli of appropriate interval completely resembles myographically the secondary contraction excited by indirect stimuli (*v. infra*) which is certainly a contracture.

When two successive stimuli fail to cause the contracture, for instance because too far apart, the addition of a third stimulus following the second at a similar, or even greater, interval, can evoke the contracture. If three do not suffice a greater number may. As is usual with "addition latente" an insufficient frequency of the stimuli can be met by increase in serial number.

Discussion.

It was not foreseen that two successive direct stimuli should, in order to be effective, require to be separated by an interval not less than the absolute refractory period of the muscle fibres as measured by the usual method for tetanic summation. It is as though the contracture and the contraction, though very different in their speed of excitation, were yet linked by a refractory period common to both. During the absolute refractory period the muscle, although it retains the effect of the first stimulus in the form of "excitation latente," appears insensitive to the second stimulus. The functioning of the muscle fibre is thus singularly complex.

The ineffectiveness of the single break shock for producing contracture in the above experiments seems to contradict the well-known fact that it is possible to evoke contracture of the veratrine type by single induction shocks of high intensity, or by brief rectangular currents of high voltage [Lapicque and Weill, 1912]. The contradiction is merely in appearance. The contracturing power of these brief currents is due to their intensity being such as to do damage [Suranyi, 1926]. In my experiments the short currents (induction shock, condenser discharge) were of physiological intensity and just supramaximal; they never gave contracture. I cannot then agree with Lapicque [1929, p. 105] who regards brevity of electrical stimulus as efficacious for causing contracture.

The observations of M. Lapicque and J. Weill [1912] are capable of another interpretation. In their experiments stimuli of extreme brevity, rectangular currents of $0{\cdot}1\sigma$

for the rectus anterior of *Rana*, gave contracture; the explanation lies in the considerable intensity required to be used in order to obtain twitches of appreciable height. The contracture was certainly a Tiegel contracture, as Gasser [1930] has suggested. The rectangular currents of longer duration (1–2σ), but yet far below the chronaxie of contracture, did not give contracture because their intensity was no longer harmful, and their duration insufficient to reach (physiologically) the threshold of contracture.

V. Neuro-muscular contracture.

Of all suggestions offered to account for neuro-muscular transmission the simplest is that which likens the motor impulse in its excitation of the muscle fibre to a brief electric current. Accepting this provisionally, and admitting that the motor nerve impulse acts with a brevity comparable to that of break shock in a coreless coil, such nervous impulses, taken singly, will be as devoid of contracturing effect as are such break shocks taken singly. But when suitably repeated they will prove effective, by "addition latente."

Tiegel [1876], and after him many observers, notably Langley [1905, 1909], were struck by the impossibility of evoking a contracture by the single indirect stimulus, even of supramaximal intensity. Beritoff [1923] claims to have sometimes excited a veratrine-like contracture by a single-shock indirect stimulus to gastrocnemius (*esculenta*), the frogs having been at 22° C. for several days. I have never, myself, in hundreds of experiments, obtained a contracture in gastrocnemius from one single-shock indirect stimulus alone.

Of course, "contraction remainder," such as occurs after any shortening, active or passive, in muscles lightly weighted under fatigue, or even fresh as in the case of ileofibrilaris and coraco-brachialis of frog [Sommerkamp, 1927] is not counted as contracture. These residual shortenings, due to muscular viscosity, can, it is true, vary in the same sense as tendency to contracture; but they are not themselves true contracture, *i.e.* an active reaction. I have to insist on this distinction, since Wachholder [1931] has recently adduced as evidence of a contracture excited by a single indirect stimulus a myogram typically that of contraction remainder in coraco-brachialis. This pseudocontracture from single indirect excitation differs from the neuro-muscular contracture just described, not only myographically, but has none of its typical features, notably not the characteristic fatigability.

I find, however, perfectly possible the regular excitation of contracture in the frog by summation ("addition latente") of nerve impulses. The conditions are those given above in the previous section, with the one difference that the stimuli are applied to the sciatic nerve of the normal frog instead of to curarized muscle. The sciatic is cut at the level of the plexus and armed with Lapicque electrodes (Ag—$AgCl_2$) half up the thigh, cathode distal.

The slow "secondary contraction" provoked by two successive in-

direct stimuli at appropriate intervals is just like that provoked by two direct stimuli. The height of the isotonic myogram is similarly a function of the interval between the stimuli (Figs. 5, 6, 7 and 11). The slow contraction as a rule appears (Fig. 5 C) so soon as the interval exceeds the

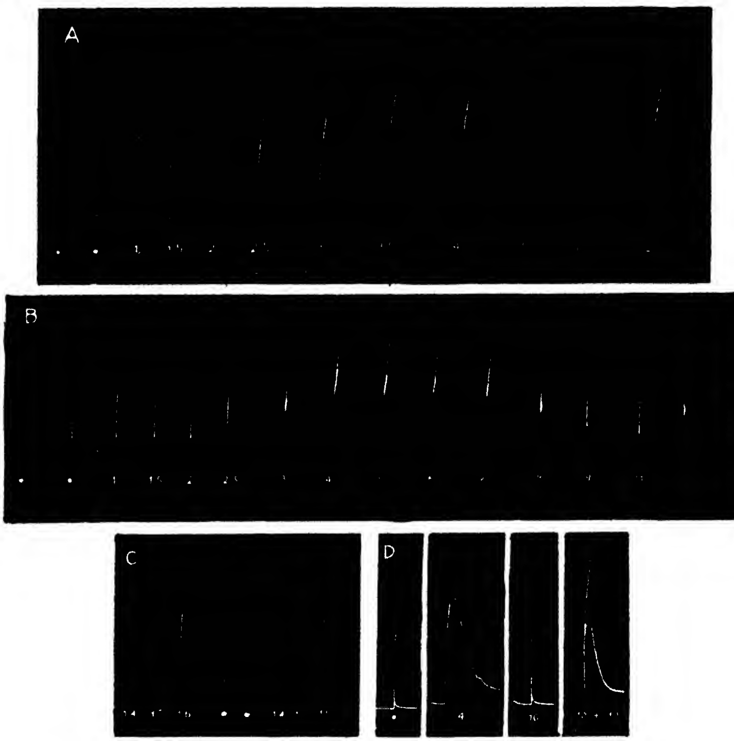


Fig. 5. Neuro-muscular contraction (isotonic) of the gastrocnemius of *R. temporaria*. Time in seconds. Reduced by ½. A. Nerve and muscle at 18° C. B. Nerve at 18°, muscle cooled to about 10° C. C. Another preparation showing the coincidence at about one ten-thousandth of a second, of the minimum interval for summation of contracture with the minimum interval for tetanic summation. D. Another preparation showing the contracturant efficacity of three successive stimuli, when two are ineffective because of too great an interval (10σ).

absolute refractory period of the motor nerve fibres ($\pm$ $1{\cdot}5\sigma$ at 18° C.). Its height increases rapidly with increase of the interval up to 3–4σ, then gradually declines. Usually all trace is lost at intervals above 10σ (Figs. 5 A and D, 7 and 11). The rise to the optimum (Fig. 6) recalls that of the curves of impulse summation (*addition latente périphérique et*

centrale), which I have described and analysed elsewhere [Bremer, 1927, 1929 *a*; Bremer and Homès, 1932].

The position of the optimal interval depends on the temperature of the muscle (and neuro-muscular junctions) and is independent of the temperature of the nerve at the seat of stimulation (Figs. 5 A and B, and 6). Conversely, the minimal interval for summation depends, *ceteris*

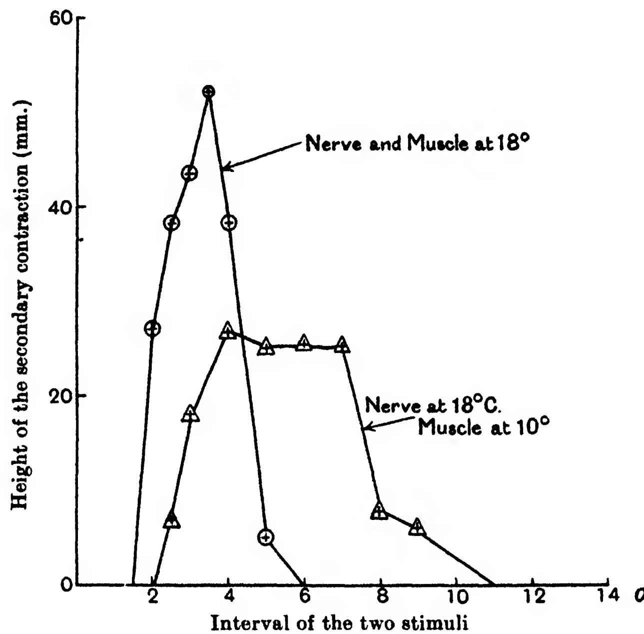


Fig. 6. Summation curves for neuro-muscular contracture constructed from the series of twitches A and B of Fig. 5. Notice the displacement toward the right of the optimum interval for summation, resulting from cooling the muscle (curve with triangles).

paribus, on the temperature of the nerve. As usual with "addition latente" it is possible to compensate excessive separation of the stimuli by adding to the serial repetitions (Fig. 5 D).

Myographic characters of neuro-muscular contracture.

The features of the slow "secondary contraction" prove it a contracture and not a contraction of special muscle fibres. Thus, it can be added to the residual contractions of fatigue or to acetylcholine contracture. In some instances I have succeeded in exciting strong contracture in a perfectly fresh muscle. There (Fig. 7) the line of the myogram joined the

abscissa in some seconds without trace of permanent residual contraction. Conversely, when excited in a muscle which has previously for a short time been tetanized, it revives somewhat the residual contracture (of fatigue), and there occurs a progressive rise of the abscissa when a succession of twitches followed by secondary contraction are evoked (Fig. 11 A). It is the secondary contraction which revives the residual

Fig. 7. Neuro-muscular contracture (isotonic) of a fresh gastrocnemius of *R. temporaria*, without any preliminary tetanization. Temp. 17° C. Reduced by ½.

Fig. 8. Acetylcholine contracture and neuro-muscular contracture. (Gastrocnemius of *R. temporaria in situ*, acetylcholine injected intravenously.) Neuro-muscular contracture accentuates acetylcholine contracture when the latter is in the ascending phase, while it accelerates the annulment of alcaloidal contracture in the regressive phase even more than do ordinary twitches. Reduced by ⅓.

contracture because simple twitches and tetani not followed by secondary contraction do not do so (Figs. 5 and 11). In the same way as intercurrent neuro-muscular contracture hastens the onset or increases the intensity of acetylcholine contracture (Fig. 8).

The height and shape of the isotonic myogram of the secondary contraction vary from preparation to preparation, but for the same experiment are constant. The height of the secondary contraction in a muscle lightly weighted (5 g.) can much exceed the height of a brief tetanus (Figs. 5 A, 9 A and 10 C). Again, the secondary contraction can graft itself

so early upon the twitch that it appears merely as an increase of height and duration of this latter. In general its form is that of a slow smooth twitch lasting several seconds (Figs. 4 A, 5, 6 and 7). At times the relaxation is late and irregular (Fig. 9 A); in such cases inspection finds the muscle bunched up towards its proximal (neural) pole and fibrillating more or less markedly.

The tension of the secondary contraction is always less than that of the twitch which precedes it, even when the shortening of the contracture

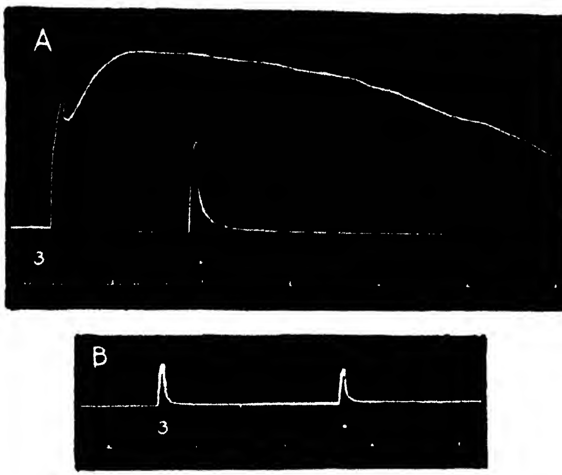


Fig. 9. Twitches followed by a secondary contraction and single maximal contractions isotonically recorded (A), and isometrically recorded (B), at an interval of a few minutes. An extreme example of the contrast existing in neuro-muscular contracture between the degree of shortening of the muscle and the feebleness of the tension developed by the contracture. Time in seconds. Reduced by $\frac{1}{3}$.

(isotonically registered) exceeds that of the twitch (Fig. 9). The strongest secondary contraction I have registered has not exceeded $\frac{1}{5}$ tension of the corresponding double twitch. This disparity between shortening and tension is often met with in contracture both of skeletal and of smooth muscle. It is important to bear this in mind in relation to the metabolism of contractures such as acetylcholine contracture. A contracture which is strong to judge by the isotonic record of a lightly weighted muscle may develop little contraction tension, and give correspondingly little lactic acid or heat. This has to be remembered of the recent experiments of Miura [1931] and of the interpretation he gives.

Fatigue and temperature on neuro-muscular contracture.

Neuro-muscular contracture is incomparably more sensitive to fatigue than is the neuro-muscular twitch. A rapid succession of twitches, each with its appended secondary contraction, soon tires out the latter

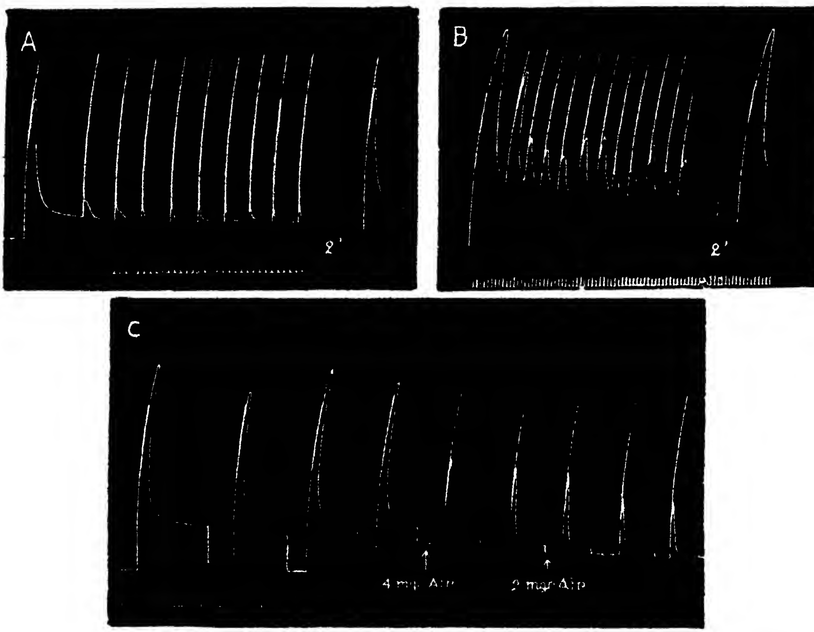


Fig. 10. A. Fatigability and very rapid restoration of neuro-muscular contracture. Having disappeared completely after a rapid series of 10 contractions (double tetani at an optimum interval of 4σ), it reappeared with the same intensity after a rest of 2 min. B. Lesser fatigability of contracture provoked by the summation of two direct stimuli on a curarized muscle. C. Selective sensitivity of neuro-muscular contracture to atropine. Double tetanic contractions (optimum interval $3{\cdot}5\sigma$) elicited every 2 min. Two successive intravenous injections of 4 and 2 mg. Atropine sulphate considerably weakened the secondary contraction without diminishing the height of the twitch. On the three records, time in seconds. Reduced by $\frac{1}{3}$.

(Fig. 10 A). Some minutes of rest restores the contracture fully. The fatigue seems mainly seated at the neuro-muscular junction, for contracture by summation of direct stimuli (Fig. 10 B) is more resistant. Besides fatigue of neuro-muscular contracture ensues quicker from twitches followed by contracture than from twitches not so followed. The mechanism of neuro-muscular contracture is therefore fatigable through the action of rapid contraction but still more so through its own action.

Warming of the muscle to 25° C. or higher annuls at once its tendency to neuro-muscular contracture (Fig. 11 A, B), but the contracture response to direct galvanic stimulation persists. Cooling the muscle restores ability for neuro-muscular contraction (Fig. 11 C).

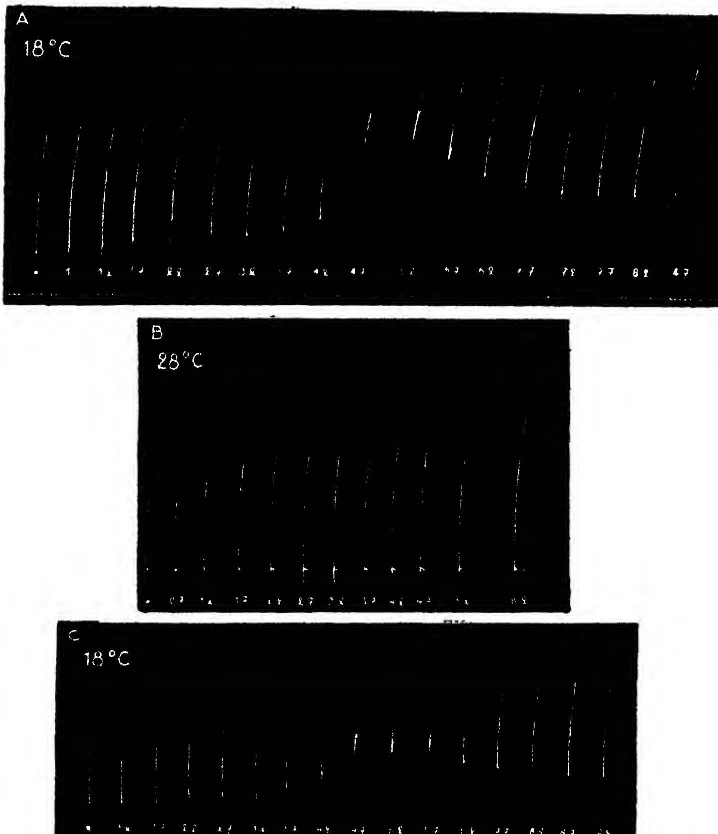


Fig. 11. Reversible disappearance of neuro-muscular contracture by warming the muscle to 28° C. The three series of twitches were successively recorded in the course of half an hour. Time in seconds. Reduced by ½.

Pharmacology of neuro-muscular contracture.

Contracture is somewhat selectively affected by curare. Under incomplete curarization the elicitation *via* the nerve of the rapid twitch is always less impaired than is that of the "secondary contracture" (Fig. 4 A). This is intelligible, since the production of the contracture,

involving a summation of impulses, is more complex and thence more vulnerable than that of the neuro-muscular twitch. At the stage in which contracture is abolished the twitch is however much reduced (Fig. 4 A).

The atropine group has a markedly selective action on neuro-muscular contracture, even in doses which do not touch the twitch (Fig. 10 C). The atropinized muscle still responds by contracture to suitable direct stimuli (Fig. 4 D). There seems in fact a true "curarization" of the contracture, a point of interest for its explanation. The dose of atropine abolishing neuro-muscular contracture is much higher than that suppressing the parasympathetic effects. This action of atropine is probably unrelated to its parasympathetic affinity. Suffice it here to cite the identity of refractory period of the "motor" fibres of contracture and of ordinary motor fibres (Fig. 5 C). Further, the lack of proof, anatomical and pharmacological [Dale and Gasser, 1926], of any parasympathetic innervation of the skeletal muscular fibre.

As already stated the neuro-muscular and acetylcholine contractures sum one with another (Fig. 8). It is, however, little likely that acetylcholine plays a part in the production of neuro-muscular contracture, which exhibits only exceptionally the "resolution" [Riesser, 1921, 1925; Hess and Neergaard, 1924] so characteristic of choline contracture (Fig. 8). Further, acetylcholine in the perfusion fluid (oxygenated Ringer) for the gastrocnemii when they are giving strong neuro-muscular contractures gives me wholly negative results in the five experiments I have performed. Hearts of *R. temporaria* used as reagents for the alkaloid were sensitive to dilutions of acetylcholine of 10^{-7} to 10^{-9}.

Adrenaline, even in toxic doses (0·01 mg. per g. intravenously injected), is without effect on neuro-muscular contracture. I have performed no experiments using ergotamine.

Discussion.

To obtain a contracture of skeletal muscle by indirect excitation, it is thus necessary to make use of the mechanism of summation of nervous impulses by repetitive stimuli. This neuro-muscular contracture furnishes a myogram exactly similar to that of the summation of two or more brief stimuli applied to the same muscle after curarization. These facts are in perfect accord with the hypothesis that reversible contracture of skeletal muscle is the reactional expression of a very slow, specific excitability of the muscle fibre.

The remarkable pharmacological properties of neuro-muscular contracture seem to me to furnish a new argument in favour of this conception.

As we have seen, there is a manifest difference between the action of curare and of atropine on slow secondary contraction, in respect of their selectivity: when applied to the gastrocnemius of *R. temporaria*, atropine and scopolamine paralyse selectively neuro-muscular contracture, while the action of curare is on the contrary but very imperfectly selective.

In the theory of Lapicque [1926], taken in its most general form, curarization of a neuro-muscular apparatus is considered to result from the rupture of the minimum accord necessary for neuro-muscular transmission between the respective speeds of excitability (chronaxies) of the muscle and the nerve. This theory of curarization could be further enlarged by attaching it to the general hypothesis of Herman-Cremer-Lillie, and expressing it thus: The abolition of nervous command over a muscle results from all alterations of the excitability of the muscle fibre which render it inexcitable by those particular stimuli constituted by the action currents of the nerve. This alteration of excitability may be either a modification of the chronaxie, or a simple rise in the threshold of the muscle fibre.

It is well known [Lapicque, 1926] that atropine and the alkaloids of its group are much more toxic for slow muscles than they are for rapid muscles, while exactly the opposite is found in the case of curare. This explains why atropine, which has but an imperfect effect on rapid muscles, has in sufficient doses an effective curarizing effect on slow muscles. Its selective curarizing action on neuro-muscular contracture is thus well explained by the hypothesis that this contracture is the manifestation of a very slow excitability which is as such very susceptible to atropine. The possibility of this selective curarization of contracture may be considered a proof of its long chronaxie. The reality of a specific toxic action of atropine on the excitable substance of contracture is further demonstrated by the fact that this alkaloid, when in a much higher concentration than that necessary for paralysing neuro-muscular contracture, also causes the disappearance of galvanic contracture in the muscles of Anura, without nevertheless abolishing the ordinary rapid contractility [Rückert, 1930 *b*].

VI. Electromyogram of neuro-muscular contracture.

The electromyographic study of neuro-muscular contracture is of particular interest. The experiments that have just been described tend to show that reversible contracture of skeletal muscle is the expression of a specific type of excitation of the muscle fibre. At present it seems well established that the electrical manifestations of muscular contraction are, in part at least, determined by the state of excitation of the muscle fibre, independently of the mechanical phenomenon of contraction. Bishop and Gilson [1927], confirming the older observations of Lee [1887], have shown, in fact, that the monophasic electrogram of the isometric twitch of the frog's sartorius is the expression of an electrical negativity lasting during the entire period of contraction, but that this electrogram might be dissociated to a certain extent into two successive phases: (1) a brief initial negative phase of great amplitude; (2) a second negative phase, normally more or less fused with the first, the shape of which corresponds to that of the isometric myogram. According to Bishop and Gilson, the first phase is the electrical expression of the wave of excitation passing over the fibres, while the second corresponds to the electrochemical reaction which is supposed to be the basis of the process of contraction. As Gasser [1930] suggested, one can equally well account for this division of the electromyogram into two successive elements by assuming that the muscle fibre in a simple contraction passes successively through two distinct, although causally linked, stages of excitation: (1) the wave of excitation and of negativity which is translated into the initial sharp deflection, and (2) the state of excitation which is the immediate cause of the contraction, and which is shown by the delayed phase of negativity.

If this dualistic interpretation of the electromyogram is correct, then reversible contractures, which are characterized by the absence of propagation of the contractile process along the fibre, should present as their sole electrical manifestation only that negativity which is in immediate causal relationship with the contractile process and the intensity and duration of which vary proportionally as the intensity and duration of the latter. This functional negativity ought to evince itself, in the monophasic electromyogram, in a curve devoid of oscillations, whose shape corresponds more or less to that of the isometric myogram of the contraction, but which may be initiated before the appearance of tension.

This is essentially what has been observed in the case of many reversible contractures. But these potentials have been very differently

interpreted: in some cases as the electrical manifestation of an authentic state of excitation, in others as a current of deformation, and in still others as a current of alteration resulting from the harmful action of the chemical agent producing contracture [see literature in the review of Gasser, 1930].

Further, the presence of rapid oscillations complicating a continuous deviation in the electrogram of various contractures, notably of veratrine contracture, has often been interpreted, recently by Wachholder [1930 *a*], as the proof of the fundamentally tetanic nature of these contractures. To these conclusions Dittler and Freudenberg [1923] and Gasser [1930] have raised serious objections.

All these electrographic studies were made, besides, on chemical contractures, capable of causing changes in the muscle which in themselves might generate differences of potential. Thus it was particularly interesting to make an electromyographic study of a contracture provoked by electrical stimuli, especially indirect stimuli, which joins to the advantage of being the most physiological method, that of eliminating all possibility of an electrical artefact due to the stimulus.

Beritoff and Woronzow [1926] have already studied the electrical manifestations of the post-tetanic contracture of the gastrocnemius of the frog indirectly faradized. They demonstrated the existence of a non-oscillatory negativity concomitant with the contracture, which is gradually dissipated in the same time as the latter. But it is probable, because of the conditions (prolonged tetanus) and the object (*R. esculenta*) of their experiments, that the contracture was one of fatigue and not the contracture by excitation in which we are interested.

The neuro-muscular contracture of *temporaria* lends itself well to electromyographic registration because of its constancy of reaction, which permits the successive registration, in a few minutes, of twitches followed by a secondary contraction, and of ordinary single twitches.

These researches were performed by means of a Cambridge string galvanometer. Because of the small size of the electromotive forces involved the tension of the string was in general reduced to a displacement of 30–40 mm. per millivolt, which, considering the optical amplification and the resistance of the string according to the formula of Forbes and Ray [1923], represented a sensitivity of 330–450 metres per ampere. The leading electrodes were two fine silver chloride pins, one of which was inserted into the belly of the gastrocnemius muscle, and the other into the tendon of Achilles, which was itself attached to a Sherrington isometric myograph arranged for optical registration. The

resting current, generally feeble (several millivolts), was always compensated.

My own observations of the electromyogram (Pl. I) of twitches free of contracture registered under these strictly isometric conditions, with approximately monophasic derivation, and with a high string sensitivity, find that it is composed of two successive and clearly distinct phases, fully confirming the results of Bishop and Gilson [1927] mentioned above. In the case of double tetanic contractions, provoked by two stimuli at a sufficiently long interval, the positive appendage of the initial negative deflection is double (Pl. I C), while the delayed negative deflection remains simple corresponding to the myogram of the two fused contractions.

This duality of the monophasic electromyogram of a twitch, recorded with a galvanometer having a very sensitive string, corresponding to that which Bishop and Gilson [1927] obtained with the cathode-ray oscillograph in their study of the monophasic myograph of the isometric twitch of the frog's sartorius is, seemingly, as these authors suggested, the expression of a duality of origin of the potentials. The brief initial phase represents, according to this hypothesis, the potential of the muscular "impulse" which precedes the contraction; the second phase, of lower potential, and which is synchronous with the development of tension, would be intimately associated with the process of contraction. Control experiments permitted the exclusion both of the phenomenon of De Meyer (current of deformation), and of an artefact resulting from displacement of the electrodes. Pl. I D shows that passive variations in the tension of the muscle, although greater than the active variations corresponding to contractions A, B, and C, cause but insignificant variation in potential when compared with those of the delayed phase of the electromyogram of these twitches.

The fundamental experiment destined to demonstrate the electrical sign of neuro-muscular contraction consisted in recording, in a rapid series, the isometric myogram and electrogram of four twitches, some (the 1st and 4th, Pls. II and III A and D) followed by the slow secondary contraction because they were promoted by two indirect stimuli of adequate interval ($\pm$ 3·5σ), and others (the 2nd and 3rd, Pls. II and III B and C) free of contractures because they were provoked by a single stimulus, or by two stimuli at a sufficiently great interval (in general 10σ). As in my preceding experiments, the tendency of the muscle toward neuro-muscular contracture was augmented by a short tetanization preliminary to the rest of the experiment.

PLATE I

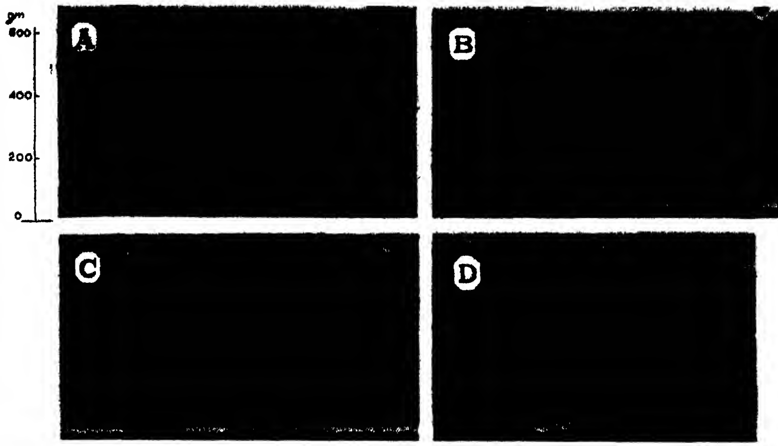


PLATE II

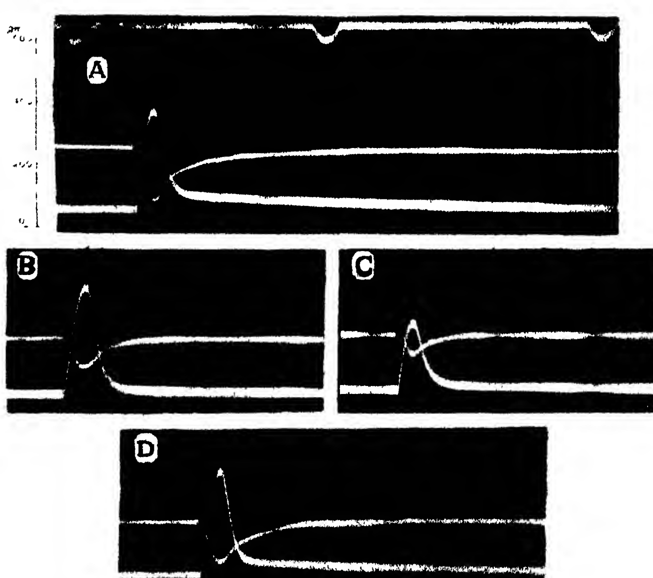
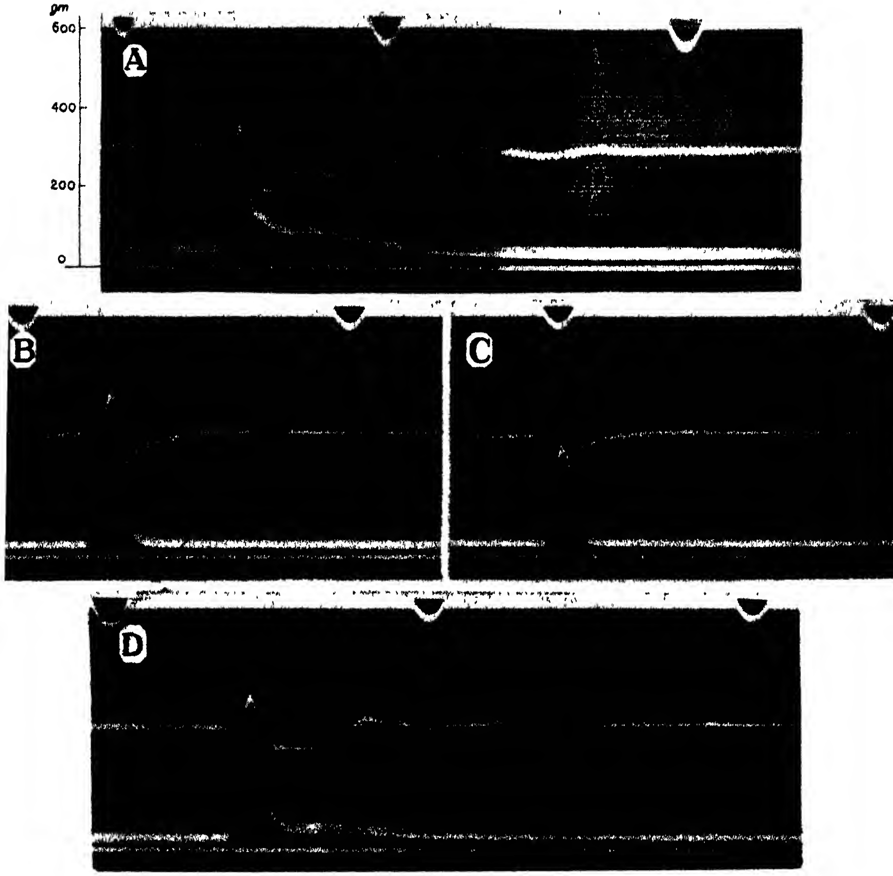


Plate III



The neuro-muscular contracture is represented on the isometric myogram (Pls. II and III A and D) by a renewal of the contraction which interrupts at various points the phase of relaxation of the double tetanic contraction, and which lasts, although progressively decreasing, for 1–2 sec.

Electrographically (Pls. II and III A and D) the contracture is characterized (1) before any mechanical manifestations by the augmentation of the amplitude of the delayed negative wave of the electrogram of the twitch; (2) by the prolongation of the decreasing phase of this wave in a curve which slowly approaches the line of isoelectricity at the same time as the line of the myogram approaches the position of initial tension of the quiescent muscle. The parallel between the two curves is striking. Sometimes the line of the electrogram of the contracture is irregularly scalloped (Pl. III A and D). These waves may pass the line of isoelectricity, especially near the end of the contracture, and give the appearance of a diphasic electrogram. In general, with the exception of these apparently secondary waves corresponding to the waves in the myogram, the curve is simple. But from time to time one can distinguish very fine rapid oscillations which are hardly visible. The appearance of the electromyogram of contracture is somewhat variable from one experiment to another, but is remarkably constant for the same muscle and for the same position of the leads.

The negative potential associated with neuro-muscular contracture is slight, as is the tension developed by the contracture, and is at all times proportional to this tension. The highest electromotive force that I have recorded is of about 3 millivolts; it corresponded, in this experiment, with a tension of 40 g., while the tension of the contraction which it followed was 300 g.

Discussion

From these observations it seems that the monophasic (from muscle-tendon leads) electromyogram of neuro-muscular contracture of the gastrocnemius of *R. temporaria* consists essentially of a non-oscillatory negativity of the active lead. This negativity, being neither current of deformation, as demonstrated by control experiments, nor a result of alteration of the muscle substance, because the contracture was provoked by indirect stimuli, can be legitimately considered as the electrical expression of the specialized type of muscular activity represented by contracture. This potential is feeble, as is the tension developed by the contracture itself, is proportional to this tension and is slowly dissipated in the same as this latter.

The small rapid oscillations sometimes present in the electromyograms are seemingly the electrical sign of fibrillations which are visible to the naked eye in the muscle in contracture.

These observations and these interpretations are in complete accord with electrographic observations which have been recently made on other reversible contractures in skeletal muscle, especially with those of Schäffer and Licht [1926 *a*, *b*] on acetylcholine contracture and the Vulpian-Heidenhain phenomenon, and of Bishop and Kendall [1929] on aldehyde contracture of the frog's sartorius. Shäffer and Licht demonstrated the complete parallel between the electrogenic activity of acetylcholine and its contracture producing action (experiments on the mammalian muscle, in a normal state or in a state of Wallerian degeneration), while Bishop and Kendall discussed, and rejected categorically, the opinion of Verzár and Peter [1925], which attributes the potential changes in aldehyde contracture (which is of the veratrine type), to alterations contingent on the form and electrical resistance of the muscle in contracture.

My experiments, performed under strictly isometric conditions which in themselves exclude the above interpretation, reveal an additional fact, irreconcilable with that interpretation, namely, that the negativity associated with the contracture commences several hundredths of a second before the appearance of the tension on the simultaneously recorded isometric myogram. One sees in Pls. II and III that the amplitude of the negative phase corresponding to the period of relaxation of the twitch is clearly greater in the record of twitches A and D, which are followed by a contracture, than in twitches (single or double) in which contracture is absent. This increase of negativity does not correspond to any increase in contractile tension, for the tension developed by neuro-muscular contracture never determines an increase in the height of the isometric twitch. Yet it is probable (see Fig. 2 B) that the period which immediately precedes the appearance of the contracture is already characterized by an increase in the muscular viscosity.

General discussion and conclusions.

The aim of these researches was to verify the hypothesis, already deduced by a critical examination of known facts, that reversible contracture of skeletal muscle is the expression of a particular type of excitation of the muscle, which is characterized by the slowness of its development, and especially of its dissipation.

In favour of this conception I may claim to have brought forward new arguments, some indirect, and others more direct. I have been able to show: (1) that conformable to the well-known law relating the duration of the process of excitation in a tissue to the speed of excitation of the same object, to elicit a reversible contracture requires either a single stimulus of a considerable duration, or the "addition latente" of two or more stimuli which are singly too short to be effective; (2) that by using particularly sensitive muscles, such as the gastrocnemius of *R. temporaria*, one can obtain a true contracture by indirect stimulation if one takes advantage of the phenomenon of the "addition latente" of nervous impulses. I have indicated that the similarity between this "addition latente" of nervous impulses, and the "addition latente" of direct electrical stimuli of very short duration, results evidently from the fact that the bioelectric currents of the nerve impulse have the same effect on the muscle as very brief electrical stimuli; (3) a third and direct argument was furnished by a study of the electromyographic properties of neuro-muscular contracture. This contracture, provoked by truly physiological stimuli, independently of any pharmacological action, is shown to be characterized by a non-oscillatory negativity, the curve of which follows closely that of the contractile tension developed.

This particular form of excitation of the muscle may be supposed to set into action the same contractile mechanism as is employed in ordinary contraction. The identity of the metabolism of contracture and contraction demonstrated by Hartree and Hill obliges us to acknowledge the unity of the contractile mechanism. There is thus a duality of excitation but not a true duality of contraction. Contracture and contraction then, seem only to differ in the nature and duration of intermediate processes constituting the protoplasmic excitation, which form a link in the chain of phenomena leading from the stimulus to the liberation of muscular energy.

This conception of the mechanism of contracture approaches in detail that which was more or less explicitly developed by Jensen [1914], and by A. V. Hill [1926, p. 76]. That of Jensen in particular postulated, as Gasser [1930] also emphasized, "two modes of approach to the same metabolism or mechanism."

All the evidence indicates that the "excitable substance" of contracture co-exists in each individual muscle fibre with the excitable substance of rapid contraction, and is not provided for by special fibres. I have shown, in fact, that the nerve fibres responsible for the transmission of the "motor" impulses of neuro-muscular contracture are in

all probability the ordinary motor fibres. On the other hand, if it is true that different muscles of the same vertebrate can be differentiated on the basis of their unequal aptitude for contracture, and that one can even distinguish in certain muscles, as in the ileofibularis of the frog studied by Sommerkamp [1927], two groups of fibres, both on the basis of their contractile properties and their macroscopic appearance, it is also true that these fibres with particular aptitude for contracture have by no means lost their aptitude for ordinary tetanic contraction.

In a muscle capable of contracture the very same fibre can thus react according to the qualities[1] of the direct or indirect stimulus, either by a twitch or tetanus free of all contracture, or by a contraction followed by a contracture.

What is the biological significance of this dual excitability? Has it an important functional significance? *A priori* this seems hardly probable by reason of: (*a*) the extreme variability of the tendency toward contracture in the same muscle, as the gastrocnemius of Anura, according to the species, and even the individuals of the same species; (*b*) the fact that contracture of skeletal muscle does not represent a more economical mode of activity than normal contraction; (*c*) the feeble contractile tension developed by neuro-muscular contracture. No one any longer defends the hypothesis of a direct relationship of contracture with postural tonus of vertebrates.

Nevertheless, the idea of a functional rôle for this type of muscular reaction has reappeared in several recent publications. Thus Hess and Büsch [1927] have suggested its adjuvant rôle in tetanic fusion.

To this hypothesis there is the objection resulting from my own researches that at a frequency necessary for neuro-muscular contracture tetanic fusion is already complete. Besides, A. V. Hill and his collaborators have demonstrated that completion of tetanic fusion is economically provided for by the effective mechanism, common to all Vertebrata, of the prolongation of the phase of relaxation of a contraction by fatigue and by initial extension of the muscle.

Wachholder [1930 *b*, *c*, 1931], demonstrating the parallel existing between the sensitivity of skeletal muscles to contracture by acetylcholine and their degree of postural activity, attributes to this fact a fundamental biological importance.

[1] I call attention here to the fact that that which determines the appropriateness of a stimulus for a "slow" excitable substance is its relative duration, and not, as Bethe, Fraenkel and Wilmers [1922] mistakenly supposed, the progressiveness of its establishment. The rapidity of establishment of a stimulus always favours its efficacity, but slow tissues tolerate a more gradual establishment of a stimulus than do rapid tissues [Lapicque, 1926].

Since mechanical, thermal, and chemical stimuli are always of considerable duration in comparison with the duration of an induction shock, their efficacity in provoking contracture can readily be explained.

But the recent researches of Freund and Rückert [1930] and Rückert [1930 *a*, *b*] lead to a different interpretation of these "tonic" properties of skeletal muscle. They have shown that the aptitude of a muscle to electrical or alcaloidal contracture varies inversely as the ontogenetic and phylogenetic evolution of the muscle, other conditions being equal. One can discover in the adult the stages of this evolution, either in comparing in the same animal muscles of different phylogenetic antiquity, or in following the same muscle through the vertebrate series. This evolution may be accelerated because of functional causes, as in the case of the wing musculature of birds, and of some of the muscles of the posterior limbs of Anura [Freund and Rückert, 1930]. The same cause, participation in rapidly alternating movement of which the execution would be hampered by contracture, seems to me to explain the striking difference in aptitude for contracture between the gastrocnemius of *R. temporaria* and *Bufo* which are terrestrial Anura whose locomotion is chiefly by walking, and the same muscle of *R. esculenta* which is a swimming and jumping animal (Fig. 12). In this sense was taken the statement of Freund and Rückert, contrary to the interpretation of Wachholder, that "only the loss of the tonic properties seems to be dependent on the functional specialization of the muscle."

Aptitude for contracture would thus characterize a primitive evolutionary state of the muscle fibre, a condition which has been passed by some muscles but not yet by others, either because despite their phylogenetic antiquity they have retained primitive function (ocular muscles of mammals, Duke-Elder [1930]), or because, by reason of a too recent appearance in phylogeny, they have not yet had time to accomplish their evolution (diaphragm of mammals).

This evolutionary stage is also reproduced by all mammalian muscles in the course of their ontogeny [Rückert, 1930 *a*]. Normal muscles of adult mammals have practically lost these "tonic" properties, but not entirely, as is demonstrated by the greater duration of galvanic contractions compared with contractions of the same height provoked by short stimuli or indirect stimuli (compare A and F in Fig. 3), and by the possibility of provoking galvanotonic contracture by very intense stimuli. The tonic properties reappear in various pathological conditions: Thomsen's disease, myotonic myopathies, and Wallerian degeneration, which are characterized by a regression to an embryonic structural type. I have elsewhere called attention to the analogies between myotonic contracture in human pathology and neuro-muscular contracture in the Anura. This comparative study has led me to the conclusion that the myotonic rigidity which prolongs contraction in Thomsen's disease and myotonic myopathies (Fig. 13) is a neuro-muscular contracture of abnormal muscles, provoked by a summation of normal nerve impulses [Bremer, 1929 *b*; Bremer and Mage, 1929].

This relationship of contracture to the evolutionary stage attained by the muscle fibre is well in accord with the facility with which smooth muscle responds to various stimuli by contractures, which in certain cases at least [Bozler, 1930 *a*, *b*] entail a notable expenditure of energy and thus cannot be interpreted simply as a manifestation of extreme muscular viscosity.

It fits in well also with the characteristic properties of the process of excitation which is the basis of contracture: slowness of development

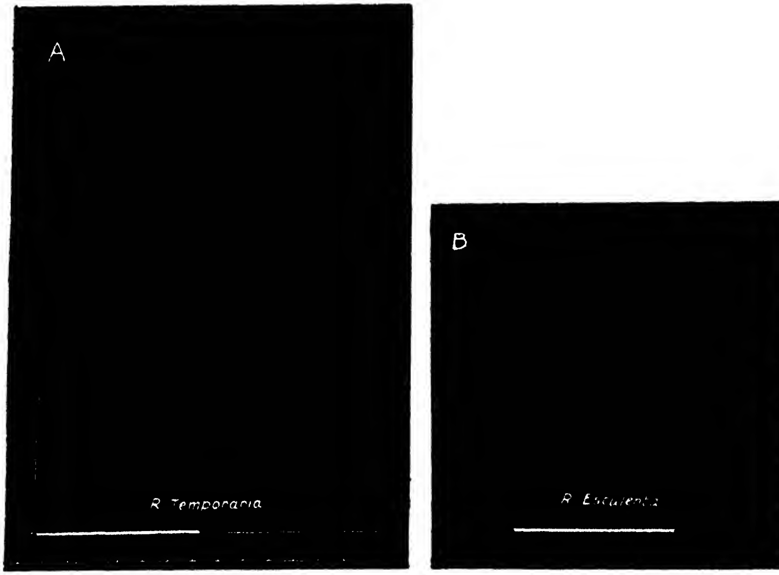


Fig. 12. A. Isotonic neuro-muscular tetanus (5 g. load) of a brown frog (*R. temporaria*) showing strong neuro-muscular contracture. Note the normal start of the plateau of the tetanus (latent period of contracture) and the dome which the contracture then produces. B. Neuro-muscular tetanus identically registered and provoked—at the same frequence of about 60 break shocks per second—of the gastrocnemius of a green frog (*R. esculenta*) of the same weight and the same state of nutrition, at the same temperature, 18° C. Time in seconds. Reduced by ½.

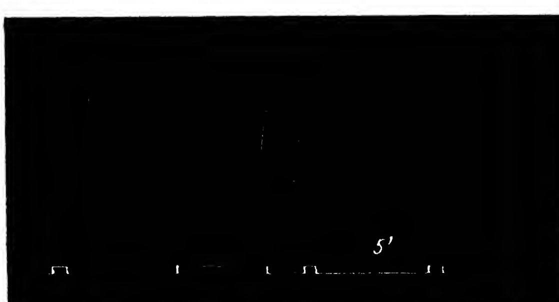


Fig. 13. Myotonic rigidity in man. Patient, 39 years, showing an accentuated myotonic myopathy. Myograms of the superficial flexors of the fingers. Registration by means of a "cardiographic" pneumograph applied over the muscle. Faradization of the motor point at a frequence of 40 break shocks per second. Note the prolonged contracture which follows the first tetanus, its disappearance after a more prolonged faradization, and its complete reappearance after a rest of 5 min. Time in seconds. Reduced by ½.

and dissipation, absence of propagation along the fibres, polar localization (cathodal), and absence or feeble development of the phenomenon of adaptation. These characteristics of the process of excitation in contracture present an analogy, already noticed by Biedermann [1895], with those of the visible reactions to galvanic stimuli by the undifferentiated protoplasm of numerous Protozoa.

Reversible contractures of the skeletal musculature of vertebrates which might be provoked by electrical, alkaloidal, or nervous stimuli, seem thus to be a primitive form of reaction of the muscle fibre. Tendency to contracture is the expression of a vestigial property, seemingly without any functional rôle, and of variable intensity according to the evolutionary stage of the muscle, its state of nutrition, and its functional specialization.

Summary.

1. The gastrocnemius of *R. temporaria* presents a particular tendency to contracture by direct or indirect electrical and alkaloidal stimuli. The better the state of nutrition of the muscle, the stronger is this tendency.

2. The chronaxie of contracture of the gastrocnemius of *R. temporaria*, measured by means of metallic electrodes and "longitudinal" excitation, is about a hundred times that of ordinary contraction.

3. Very brief direct electrical stimuli (for example break shocks), of physiological intensity, although maximal as far as the twitch is concerned, do not cause contractures of the curarized gastrocnemius when applied singly, but become effective only when repeated often enough and at an adequate interval, because of summation of stimuli. Two successive stimuli can suffice. In order to be effective at the ordinary temperature the interval ought to be between $\pm\, 2\sigma$ and $\pm\, 8\sigma$, with an optimum of $\pm\, 4\sigma$.

4. A single stimulus is equally ineffective in the case of indirect stimulation of the gastrocnemius of *R. temporaria*. In order to provoke a contracture of this muscle it is necessary to obtain a summation of nerve impulses. Neuro-muscular contraction provoked by two stimuli of adequate interval ($1{\cdot}5$–8σ, with an optimum of $\pm\, 4\sigma$) presents complete myographic analogy with the contracture elicited by summation of two direct stimuli on the curarized muscle.

5. Neuro-muscular contracture is selectively sensitive to (*a*) fatigue, (*b*) heat, (*c*) atropine and related alkaloids. The "curarization" of neuromuscular contracture by atropine may be explained by the particular toxicity of this alkaloid for substances of long chronaxie.

6. The monophasic electromyogram of neuro-muscular contracture consists in a non-oscillatory negativity of the active electrode. The potential developed, which is very feeble, is proportional to the tension developed by the contracture. Its appearance seems to precede on the electromyogram the first manifestation of tension of the contracture.

7. Myotonic rigidity of human pathology presents striking analogies in properties and mechanism with neuro-muscular contracture in Anura.

8. Contracture by excitation of skeletal muscle seems to be a primitive form of reaction of the muscle fibre, connected with an "excitable substance" of long chronaxie, and characterized by the slowness of development and dissipation of its excitatory process, its absence of wave-like propagation, and its persistence throughout the duration of a galvanic stimulus (absence of adaptation).

9. A comparative study of the same muscle, such as the gastrocnemius, in various Anura, suggests that the tendency of a muscle to contracture depends on its functional specialization, in the sense that habitual participation of the muscle in a rapid alternating movement (swimming and jumping) accelerates its evolution towards a stage characterized by no, or only a feeble, aptitude for contracture by excitation (confirmation of the hypothesis of Freund and Rückert).

I wish to express my sincere thanks to Prof. Sherrington for undertaking the translation of this paper and to Dr H. E. Hoff who assisted in this task.

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EXPLANATION OF PLATES.

Plate I.

Isometric myogram and electromyograms of the gastrocnemius of a frog showing no neuro-muscular contracture. Decerebrate animal. Tendon-muscle leads; proximal negativity (muscular) indicated by a downward displacement of the string. String tension: 50 mm. per millivolt; sensitivity reduced to 5 mm. per millivolt after the muscle was connected. From top to bottom; time in seconds, electromyogram, myogram. Temp. 20° C. Reduced by $\frac{1}{3}$. A. Single maximal twitch (indirect excitation). B and C. Double tetanic contractions elicited by two break shocks at intervals of $3{\cdot}5\,\sigma$ and $10\,\sigma$ respectively. D. Passive variations in tension of the same muscle.

N.B. On these records, as on those of Pls. II and III, the large initial phase of the monophasic electrogram is not seen, because of the amplitude and very great rapidity of movement of the string. One can only see the positive deflection which succeeds it. The small regular oscillations in the string record are due to induction of the alternating current of the building on the very loose string.

Plates II and III.

Isometric myogram and electromyogram of the gastrocnemius of two brown frogs showing a strong neuro-muscular contracture. Same experimental conditions as for Fig. 1. Tension of the string: 40 mm. per millivolt; sensitivity reduced to 3 mm. per millivolt after the preparation was connected. Reduced by $\frac{1}{2}$. A and D. Double tetanic contractions elicited by two break shocks of just supramaximal intensity applied to the sciatic at the optimum interval of $3{\cdot}5\,\sigma$. Neuro-muscular contracture. B. Double tetanic contraction provoked by two stimuli at an interval of $10\,\sigma$. Absence of contracture. C. Single maximal twitch. No contracture.

N.B. Contractions A, B, C, and D, were elicited in rapid succession, in this order. Explanation in text.

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EXCITABILITY OF THE SINGLE FIBRE NERVE-MUSCLE COMPLEX.

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Introduction.

The relation between the intensity of an electrical stimulus and the minimum duration for which it must be applied in order to be effective has been studied by numerous observers who have used many different kinds of muscle and various nerve-muscle preparations. The present work deals with this relationship between duration and strength of an electrical stimulus in the single fibre nerve-muscle complex. The preparation which has been used for this purpose is the retrolingual membrane in the tongue of the frog, *R. pipiens*. Data have been obtained from indirect stimulation of nerve fibres lying in the membrane as well as from direct stimulation of both normal and curarized muscle fibres. It has often been possible to make all these measurements while observing throughout the response of one and the same muscle fibre. In this respect work with the retrolingual preparation has a great advantage over the single fibre measurements of Jinnaka and Azuma [1922] and of Watts [1924], using the whole sartorius muscle, stimulated by a pore electrode.

Purpose.

The general significance of the strength-duration relationship lies in the theoretical conclusions that have been drawn from the experimental results. Since these results have been, in the main, conflicting, it is but natural that several opposing interpretations have been given to the data. The purpose of the present experiments has been to secure a direct test of the merits of two of these interpretations using a system which as far as possible avoids the sources of error that have been responsible for the conflict in previous results.

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There are two leading schools of opinion with respect to the interpretation of the strength-duration curves, one of which is that headed by Lapicque [1926]. Working on many kinds of nerve-muscle preparation, this observer finds that all strength-duration curves are identical if they are expressed in terms of certain units. According to his terminology the unit of intensity is the "rheobase," while the "chronaxie" is taken as the unit of time. Lapicque has recently produced [1931 *a*] a "canon" for the strength-duration curve scaled in these units.

Furthermore, the chronaxie plays an important rôle in the theory of isochronism which Lapicque has put forward. According to this hypothesis, the transmission of excitation from a nerve to its muscle is possible only when the chronaxies of both are approximately equal. Conversely, he believes that the blocking of such transmission is due to a condition of "heterochronism" which occurs when the two chronaxies differ by more than a ratio of 1 : 2. In the case of a curari block, heterochronism is supposedly produced by an increase in the chronaxie of muscle, that of nerve remaining unchanged.

Results in apparent conflict with this "law of isochronism" were obtained by Lucas [1907], and these have been extended lately by Rushton [1930, 1931, 1932 *a*]. Both observers used experimental conditions which are different from those of Lapicque. They find that the strength-duration curve is usually compound and can be separated intc at least two components. One of these has a very long chronaxie[1] and is called the "α-curve." The second, or "γ-curve," has a chronaxie of the order of that found on stimulation of the nerve trunk. Both Lucas and Rushton have presented evidence which indicates that the γ-curve represents the excitability of nerve, while the α-curve measures the excitability of the muscle fibres. They find, for example, that curari abolishes the γ-curve while the α-curve is unchanged. This view, therefore, denies the importance or necessity of isochronism in the normally functioning complex and generally presumes that the block in transmission produced by curari is due to an effect upon the myoneural junction.

It has already been said that these two opposing views, of Lapicque on the one hand and of Lucas and Rushton on the other, derive from conflicting experimental results. Whence does this conflict arise? There

[1] Rushton [1932] has suggested that the chronaxie of these curves, whether "canonical" or not, should be called by Lucas' term "excitation time," since Lapicque [1931 *a*] denies that it represents "true" chronaxie.

have been numerous explanations. Jinnaka and Azuma [1922] have suggested that the discontinuities found by Lucas in the strength-duration curve represent changes in the number of excitable elements responding. Davis [1923], on the other hand, found that the chronaxie of muscle increased with the size of stimulating electrode. He was confirmed by Watts [1924]. In most of their work Lucas and Rushton have used large fluid or block electrodes, while Lapicque has employed chlorinated silver needle electrodes. The compound curves may therefore be ascribed to the effect of electrode size on the strength-duration curve of muscle. Rushton [1932 *d*] subscribes to the validity of this explanation, and points out that the use of large electrodes makes it possible to bring out more clearly the difference between the excitabilities of nerve and muscle in the gross preparation. Lapicque at first [1926] discounted the results of Lucas as due to errors. In his latest papers (1931 *a*, *b*), however, he has withdrawn from this position and ascribes the results to the experimental conditions, at the same time concluding that the α-curves are "uncanonical" and do not measure the "true" excitability. Still a fourth explanation has been presented in a paper by Moore and Brücke [1931] which appeared after the present work had been completed. It will be taken up in a later section.

Now one of these explanations—that of Jinnaka and Azuma—holds that these conflicting results are due to an error introduced by measurements on a large population of excitable elements. This explanation is not without a basis of corroborating evidence from other observers. For example, Lapicque [1926] finds that the chronaxies of different nerve fibres vary inversely with the fibre diameter. Both my own experiments and those of Moore and Brücke [1931] show that individual muscle fibres may have widely different chronaxies. It therefore is quite legitimate to ask just what is represented by the averaged strength-duration curves obtained from the gross preparation.

This difficulty is obviated by the use of a single fibre preparation. Jinnaka and Azuma [1922], Davis [1923], and Watts [1924] have reported measurements of the strength-duration curves of single muscle fibres in the sartorius of amphibians. These observers have confined themselves, in testing Lapicque's theory, to a study of the effect of curari on the chronaxie of the muscle fibre. Jinnaka and Azuma, on the one hand, find that the chronaxie is increased by curarization in accordance with Lapicque's views. On the other hand, Watts finds no such change. This disagreement is possibly due to the fact that in neither group of experiments were the measurements carried out on the identical fibre

both before and after curarization. In view of the difference in the chronaxies of individual fibres either result is equally probable, and does not furnish an adequate test of the theory of isochronism.

The present work has been carried out on a preparation in which the chronaxies of one and the same fibre can be determined both before and after curarization. Furthermore, a more complete test regarding the merits of the different theories of excitation can be carried out since it is also possible to stimulate nerve fibres lying in the retrolingual membrane and thus obtain the chronaxies of both components of the nerve-muscle complex. The response used has always been "all-or-none" under the conditions of the experiments [Pratt, 1930; Gelfan and Gerard, 1930], and therefore a sharp index of response is available. Furthermore, it is possible to distinguish directly between a response due to nerve stimulation and a response caused by direct excitation of the muscle fibre. This distinction is possible because indirect stimulation through the nerve causes a contraction of the entire motor unit, while on direct stimulation only one fibre responds. The picture seen through the microscope is therefore quite different in the two cases. The distinction does not hold in the gross preparation, where a more involved analysis is employed [Rushton, 1932 *c*], in order to distinguish between responses due to direct and indirect stimulation.

Preparation.

The retrolingual membrane of the frog's tongue, a preparation which was first described by Thoma [1873], has lately been recalled to physiologists by Fischl and Kahn [1928], Gelfan [1930] and by Pratt [1930]. The single muscle fibres are widely separated from each other so that they can be stimulated individually. It is also possible, in many cases, to stimulate them through the nerve fibres lying in the membrane. Under ordinary conditions the preparations can last eight hours or more, and numerous experiments can be performed on any one particular fibre. Moore and Brücke [1931] have already reported chronaxiemetric results on this preparation. The present measurements were completed in the main before their data were published, and are obtained by the use of a different technique so that the results are therefore somewhat different. By using a modification of Moore and Brücke's technique I have been able to confirm their measurements and show the underlying cause of the differences in our results. I cannot, however, agree with their conclusions, which will be discussed in detail later.

METHOD.

The method of preparation employed is a modification of that described by Pratt and Reid [1930] which permits the use of trans-illuminated membranes with intact circulation. A longitudinal superficial incision is made in the ventral surface of the everted tongue of the decerebrate frog. The various layers of tissue are carefully cut along the mid-line without injuring the larger blood vessels. This tissue is then pinned back to expose the retrolingual membrane. The preparation is covered with Ringer's solution (modification of Stella, described by Parkinson, 1930, p. 226). A comparison of excised and intact mem-

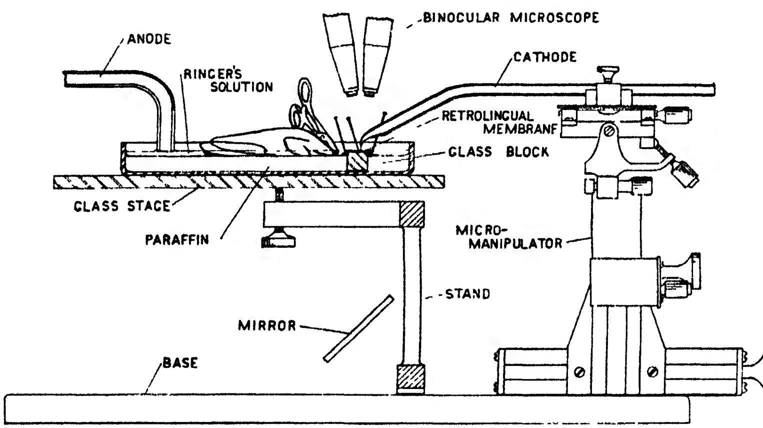

Fig. 1. Diagram of the experimental arrangement.

branes has convinced me that the latter is greatly superior, offering as it does a large number of fibres which are uninjured and therefore capable of great steadiness in threshold. Moore and Brücke [1931] make the statement that the intact preparation does not permit clear observation of the muscle fibres. In the course of my work, however, I have demonstrated the muscle fibres to many people, most of whom have had little or no training in microscopic observation and in no case has there been any difficulty in observing a response by a single muscle fibre.

Stimulation has, in general, been by means of condenser discharges, essentially as described by Lapicque. The stimuli are applied through a localized cathode which consists of a large-bore glass tube (5–6 mm. inside diameter) pulled out in a micro-flame to a capillary having an opening about 20–60 microns in diameter. This tube is filled with Ringer's

solution and is connected to a Zn—$ZnSO_4$ electrode by means of an agar Ringer's bridge. The anode is diffuse and is also made of agar Ringer's and Zn—$ZnSO_4$. The relatively large opening of the cathode assures all-or-nothing responses [Pratt, 1930; Gelfan and Gerard, 1930], while the wide spacing between the fibres of the membrane permits stimulation of only one fibre.

The experimental arrangement is easily grasped from a study of Fig. 1. The frog is placed in a petrie dish which is cemented to a movable glass stage. To the bottom of the dish is cemented a polished glass block 1 cm. square. The tongue is everted and the cut tissues are pulled out so that the retrolingual membrane lies flat on the block. The membrane is illuminated from below with the aid of a mirror and observations are made with a binocular microscope (magnification 70 ×). A layer of paraffin whose level is slightly below the top of the glass block covers the petrie dish to permit pinning the tissues. The anode dips into the Ringer's solution which covers the preparation, the cathode being held over the membrane in a micro-manipulator. By means of the various adjustments on the manipulator, the tip of the electrode is applied wherever desired.

In all the experiments which are quoted, a 10,000-ohm shunt has been used across the electrodes, which in themselves have a resistance of 150,000 to 250,000 ohms. This shunt ensures a uniform time constant for the discharge of the condensers. According to Lapicque's empirical relation between the capacity and the effective duration of the stimulus, 1·0 μF, under these conditions, is equivalent to 3·7–4·0σ, and for ease in calculation the latter value has been used throughout. A comparison between the voltage-capacity and voltage-duration[1] curves has confirmed Lapicque's relation: the two curves are identical up to a duration of about 0·1 σ (capacity 0·025μF). For times shorter than this the voltage-duration curve ascends more steeply than does the voltage-capacity curve.

Results.

The experimental results can be divided into two main sections. The first group deals with a comparison of the voltage-capacity curves for direct and indirect stimulation. The second group is concerned with the effect of curari upon the voltage-capacity curve of a single muscle fibre. Several kinds of subsidiary experiments will be dealt with after the results of the first two groups are described.

(a) *Direct and indirect stimulation of a single muscle fibre.*

In twenty-four preparations it has been possible to stimulate both nerve and muscle fibres. The results show definitely that the chronaxie is much smaller for the nerve fibres than it is for the muscle fibres.

[1] The latter have been obtained with a timing device which has been newly designed by Mr A. J. Rawson, of the Johnson Foundation. It will be described by him elsewhere.

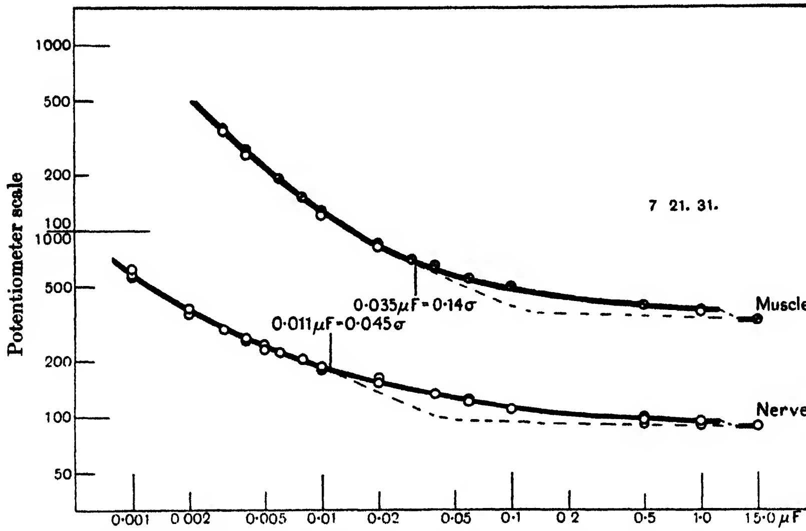

Fig. 2. Voltage capacity curves plotted in logarithmic coordinates. Ordinates: voltage in terms of divisions on a 1000-unit potentiometer scale. Abscissae: capacities in μF. The lower curve is for a single nerve fibre. The upper curve is for one muscle fibre innervated by this nerve fibre. The filled circles are measurements before curarization, while the clear circles represent measurements after curarization. The chronaxies of each tissue are given in the figure, the ratio of chronaxies of nerve and muscle fibres being about 1 : 3. The curve drawn in broken lines represents Lapicque's "canon."

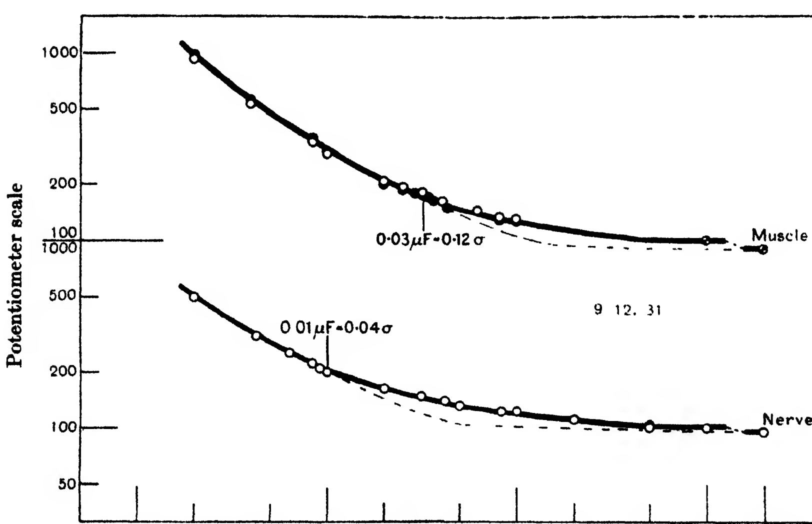

Fig. 3. Another set of curves from a different preparation.

The ratio between the two chronaxies varies from 1 : 2 to 1 : 10. Two sets of curves are shown in Figs. 2 and 3. They are plotted on a double logarithmic scale, the advantages of which have been discussed by Rushton [1931]. In each figure, the lower curve is that obtained from a nerve fibre, the chronaxie being indicated by an arrow. The filled circles in each upper curve are measurements made with direct stimulation of a muscle fibre innervated by this nerve fibre. It is seen that the chronaxies of the nerve and muscle fibres are quite different. The results of similar measurements on twenty-four preparations can be summarized as follows: (*a*) the muscle fibres have chronaxies ranging from 0·016 to 0·08μF (0·06–0·32σ); (*b*) the chronaxies found for nerve fibres vary from 0·005 to 0·013μF (0·02–0·05σ)[1]; and (*c*) the ratio of nerve-fibre chronaxie to that of muscle fibre in the same preparation varies from 1 : 2 to 1 : 10.

(*b*) *The effect of curari upon the voltage-capacity curve.*

In twelve of the above experiments curari was added to the Ringer's solution while the cathode was located upon the nerve fibre. After a few minutes, a block in transmission was established. The cathode was then replaced upon a muscle fibre whose uncurarized excitability had been previously measured. Its excitability after curarization was then determined. Figs. 2 and 3 present the data for such measurements as well. The experimental values after curarization are indicated as open circles in the upper curves. It is clear from these figures that the excitation curves and the chronaxies of muscle fibres are unaffected by curarization.

The curari used has been from three different sources, namely: (*a*) the curari "type" used at the Sorbonne and kindly furnished me by Dr Rushton; (*b*) Merck's; and (*c*) curari supplied by the firm of Eimer and Amend, New York, to the Marine Biological Laboratory at Woods Hole, Mass., where some of these experiments were carried out. The usual dosage has been a 0·01 p.c. concentration of curari in the Ringer's solution covering the membrane. Curarization is generally complete in 3 min. and very often within 1 min. Concentrations of curari as high as 0·05 p.c. have been employed without affecting the excitability or changing the chronaxie of the muscle fibres. In one experiment, a curari concentration of 0·07 p.c. caused a *decrease* in the chronaxie. This is probably ascribable to the effect of salts brought in with the curari.

These two types of experiments indicate therefore very clearly, (1) that the law of isochronism is invalid, at least for the retrolingual preparation, and (2) that Lapicque's explanation of the mechanism of curarization is incorrect, since curari does not affect the chronaxie of a muscle fibre nor does it change the excitability curve.

It is important to note that the chronaxies of the single muscle fibres are of the order of magnitude found by Lapicque [1926] on the gross preparation as well as by Jinnaka and Azuma [1922] and Watts

[1] The surprisingly small chronaxies of these nerve fibres will be discussed later.

[1924] on single fibres. Presumably, therefore, these values represent "true" chronaxie in Lapicque's newer terminology [1931 *a*]. The excitability curves do not agree with the "canon" given by Lapicque [1931 *a*] which is shown in Figs. 2 and 3 in broken lines, though it must be admitted that this "canon" was put forward in relation to strength-duration curves, and not to voltage-capacity curves.

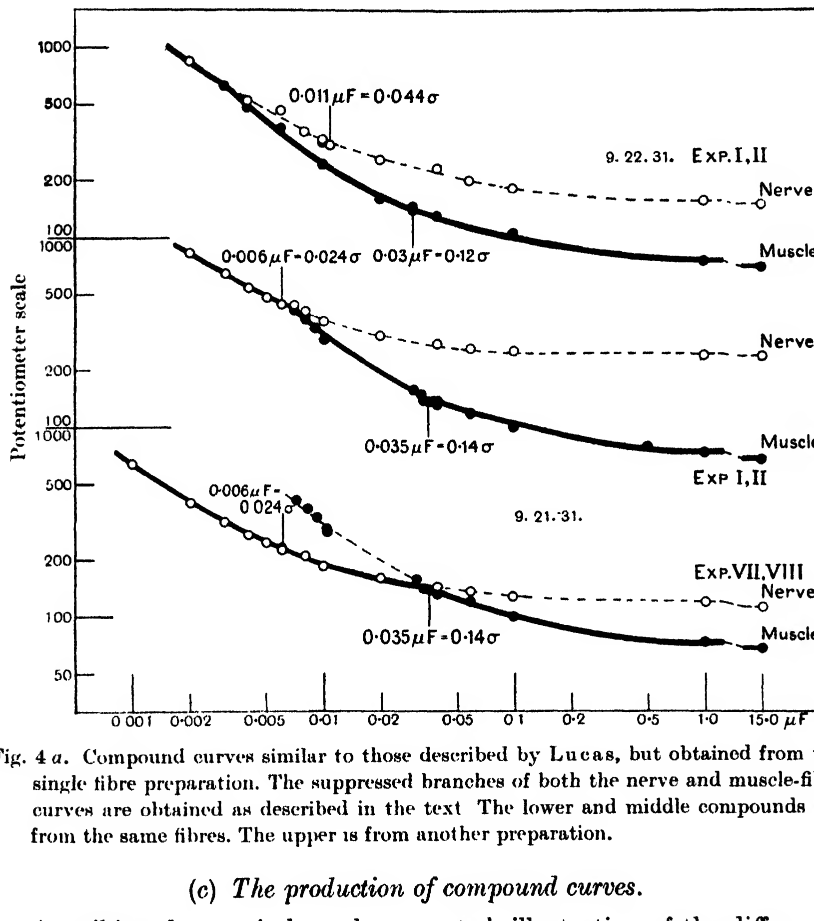

Fig. 4 *a*. Compound curves similar to those described by Lucas, but obtained from the single fibre preparation. The suppressed branches of both the nerve and muscle-fibre curves are obtained as described in the text The lower and middle compounds are from the same fibres. The upper is from another preparation.

(*c*) *The production of compound curves.*

A striking, but entirely to be expected, illustration of the difference between the strength-capacity curves of muscle and nerve fibres is shown in Figs. 4 *a*, *b*. Such compound curves are obtained in the following manner. The localized electrode is applied at a place where a muscle fibre and a nerve fibre are contiguous. Measurements are then made stimulating the muscle fibre.

It is perfectly clear from Figs. 2 and 3 that the muscle curve ascends at a faster rate than does the curve for nerve. Eventually therefore a capacity is reached for which the threshold of the nerve is below that of the muscle. The response then changes in the manner already described, there is a discontinuity in the voltage-capacity curve, and measurements are now being made along a curve representing nerve excitation. Furthermore, the lower portion of the nerve curve can be obtained by measurements which use as an index the response by the entire motor unit. This part is shown as the broken line in Figs. 4 *a*, *b*. The upper part of the muscle curve, on the other hand, can be obtained either after curarization

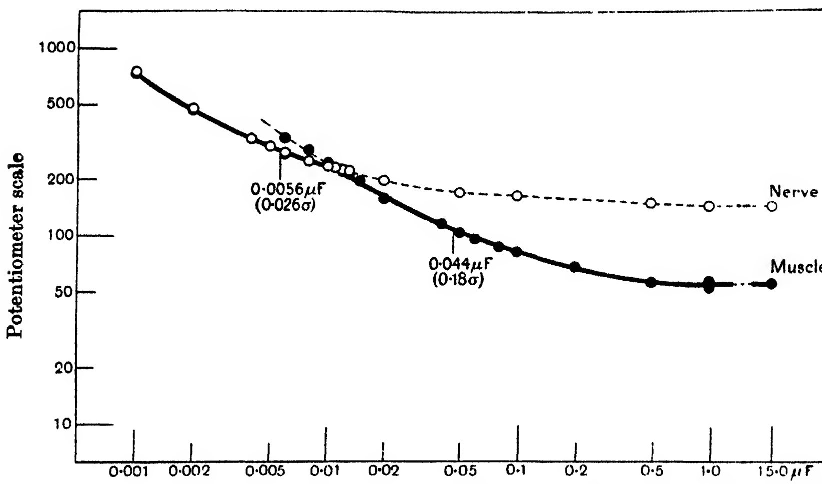

Fig. 4 *b*. A compound curve in which the chronaxies of the nerve and muscle fibres differ by a ratio of 1 : 8. The muscle fibre which was innervated by the nerve fibre had an excitability represented by the filled circles. The clear circles express the excitability of the nerve fibre. (Exp. of 3. v. 32.)

or, more simply, by moving the electrode away from the vicinity of the nerve fibre. The broken line of the muscle curve in Fig. 4 *a* (lower set) and Fig. 4 *b* represents this portion.

These compound curves, except for the different time relations, are strikingly reminiscent of the curves obtained by Lucas [1907] and by Rushton [1930, 1931]. For the moment, we shall leave open the question as to the relation which the compound curves obtained from the gross preparation bear to those found in the study of single fibres. The point which I wish to emphasize now is that in the present case, it is demonstrated directly that the composite nature of the curves in Figs. 4 *a*, *b* is due to the excitation of two different elements, namely, the muscle fibre and the nerve fibre.

The curves shown in Fig. 4 *a* also illustrate how different kinds of compounds can be obtained. The lower and middle sets are the identical fibres, but the electrode has been moved slightly so that the relative thresholds of the nerve and muscle are different. The curves therefore cross at different values of the capacity. A second way of changing the crossing point is shown in the upper set. Although the relative thresholds are intermediate to those in the other curves the crossing point comes at a much smaller capacity because the chronaxies of the nerve and muscle fibres, and therefore the forms of the strength-duration curves, more nearly approach each other.

In sum, therefore, all the results produced so far show that the single fibre nerve-muscle complex has two distinct excitabilities. Structurally, these excitabilities are represented by the two components, nerve and muscle. Furthermore, a block in transmission which is produced by curari does not alter the chronaxie, and unless the curari dose is much greater than is necessary to cause a block does not affect the excitability curve.

These results are in conflict with the theory of isochronism expounded by Lapicque. On the other hand, they are in agreement with the view, held by Lucas and by Rushton, that the excitable substances of nerve and muscle are different and that this difference manifests itself in a condition of normal heterochronism. It will be shown, in a moment, that such heterochronism can also be demonstrated in a preparation which is not covered with Ringer's solution and which is stimulated with Ag—AgCl wire electrodes. Consequently, Lapicque's latest objection [1931 *b*] to the view of Lucas and of Rushton disappears.

(*d*) *Heterochronism in preparations not covered with Ringer's solution.*

This type of experiment was made after the appearance of the paper by Moore and Brücke [1931], already mentioned. These authors use large Ag—AgCl wire electrodes for stimulating single muscle fibres in the retrolingual membrane. They also stimulate the hypoglossal nerve trunk while observing, in some cases, the response of muscle fibres. The chronaxies which Moore and Brücke obtain are much larger than those given above. Thus, they find that the chronaxies of the nerve trunk are $0{\cdot}9$–$1{\cdot}5\sigma$, while for the muscle fibres the chronaxie varies from $2{\cdot}5$ to 70σ. Moore and Brücke also report and illustrate remarkable differences between the response to direct and indirect stimulation. With the relatively large capillary electrode, I have been unable to find any such peculiarities as partial contracture and slow relaxation upon direct stimulation which are reported by these observers.

For these several reasons I have repeated their experiments, using, however, the intact preparation. This has been mounted in the chamber shown in Fig. 1, the floor of which is covered with a layer of moist filter paper (cut out to clear the glass block). To the filter paper is attached a chlorinated silver-wire anode running the length of the membrane underneath it and perpendicular to the muscle fibres. Since the glass block and therefore the central portion of the membrane is higher than the filter paper, the preparation is not covered by Ringer's solution. For a cathode I have used (*a*) either another chlorinated silver wire running parallel to

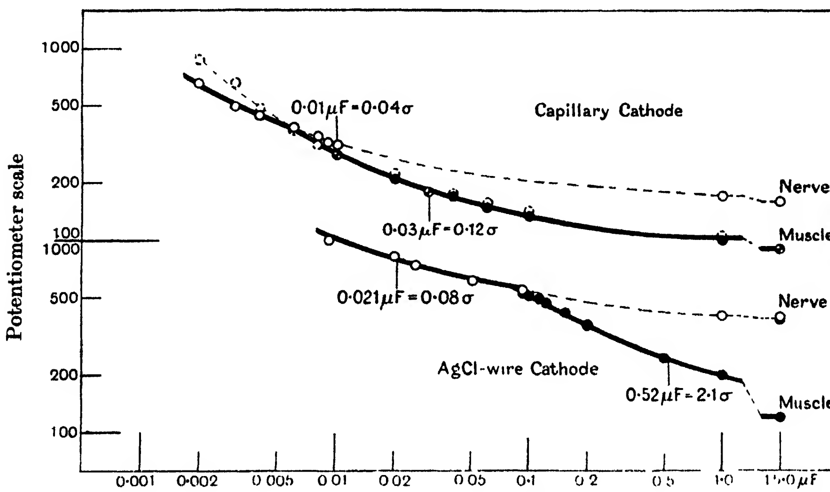

Fig. 5. Compound curves obtained from the identical fibres, with electrodes of different sizes. The lower compound has been obtained by using a silver-wire cathode, the upper with a capillary electrode. The broken circles in the upper muscle curve represent measurements after curarization.

the anode, (*b*) a silver wire bent downwards in a right angle at the tip so that only one fibre might be stimulated, or (*c*) the usual capillary electrode.

I have been able to confirm the unusual appearance of the response to direct stimulation in the case where the cathode is parallel to the anode (type (*a*)). A somewhat similar result is also obtained when two wire point electrodes are used. Responses of this type are presumably similar to the graded contractions of Fischl and Kahn [1928], Gelfan [1930], and others. However, with a cathode of type (*b*), the responses are exactly like those obtained with the capillary electrode. I am unable to explain these differences in response with the different electrode systems.

The results of measurements with cathode (*b*) and (*c*) are very interesting. They are shown in Fig. 5. The lower set of curves is obtained with a chlorinated silver-point cathode, both for a nerve fibre and for a muscle fibre. The upper set is from subsequent measurements on the same elements using a capillary cathode. The chronaxie of the muscle fibres stimulated with the wire electrode is about $2{\cdot}1\sigma$ ($0{\cdot}52\mu$F). That of the nerve is about $0{\cdot}1\sigma$ ($0{\cdot}025\mu$F). With the capillary cathode the chronaxies of the same nerve and muscle fibres are about $0{\cdot}12\sigma$ ($0{\cdot}03\mu$F) for the muscle fibre and $0{\cdot}04\sigma$ ($0{\cdot}01\mu$F) for the nerve.

This result shows quite clearly why the values of the chronaxies quoted by Moore and Brücke are higher than those obtained with the capillary electrode. The increase is due to the larger size of the electrodes which they employ. The effect of electrode size upon the chronaxie of muscle is already well known from the data of Davis [1923] and of Watts [1924]. The present work, however, is the first to show that the chronaxie of nerve is affected in a similar manner. The amount of increase is smaller for nerve than it is for muscle, but nevertheless is appreciable. The ratio of the chronaxies with the two electrode systems is 1 : 2·5 for the nerve fibre and about 1 : 20 for the muscle fibre.

TABLE I. Effect of raising the electrode above the muscle fibre.

Threshold	Chronaxie (μF)	Position of electrode
130	0·065 0·063	Touching membrane
310	0·082 0·083	Raised
480	0·14 0·13	Raised
500	0·13	Raised
200	0·085	Lowered
145	0·077	Lowered
130	0·078 0·077 0·074	Lowered
100	0·071 0·070	Lowered

The results of a somewhat similar experiment are shown in Table I. In these measurements the capillary electrode has been placed at different heights above the membrane and the chronaxie has been determined at each position of the electrode. As the electrode is raised above the membrane the area of the muscle fibre over which current is distributed becomes greater. In this respect raising the electrode is identical with an increase in the size of stimulating electrode. Column 2 of

Table I shows that the chronaxie increases when the electrode is raised and decreases when it is again lowered.

An adequate treatment of the physics of current distribution from a point electrode is beyond the scope of this work. It seems probable, however, that the change of chronaxie with electrode size can be accounted for by the resultant change in configuration of current distribution.

The results just presented have a bearing upon the compound curves of Lucas and of Rushton and their relation to the composite curves of Figs. 4 and 5. Before discussing this subject, however, two further points are of interest. Moore and Brücke [1931] conclude, from their results, that the heterochronism which they found in the retrolingual membrane complex is not contradictory to Lapicque's theory. This conclusion is based, in part, upon an argument from analogy. It has already been stated that the chronaxie of a nerve fibre varies inversely with the fibre diameter [Lapicque, 1926]. Moore and Brücke carry this datum over and apply it to muscle fibres. From this analogy they reason that heterochronism in the retrolingual membrane is due to the fact that the muscle fibres, in their experiments, are stimulated at the finely branched tips, where the chronaxie is presumed to be very large, while the nerve fibres are stimulated in the nerve trunk and therefore at their full diameter.

Moore and Brücke further apply this argument to an explanation of the curves of Lucas and Rushton. Rushton [1932 c] has answered this explanation from a standpoint which assumes that Moore and Brücke have demonstrated the dependence of chronaxie upon the diameter of the muscle fibre in the region of the cathode. Let us examine, however, the data which Moore and Brücke produce. In stimulating the muscle fibres of the retrolingual membrane, these authors use two parallel silver wires. The cathode lies clear of the fibres and near their terminations. The anode is placed upon the tongue tissue. Stimulation is, therefore, always accomplished at the tips of the fibres. Now, Moore and Brücke find that the fibres having the finest terminal branches exhibit a larger chronaxie than do the fibres ending in blunt processes. This is the extent of their proof for the assumption that the chronaxie varies inversely with the diameter of the fibre at the locus of stimulation. Using both the pointed wire and the capillary electrodes as cathode I have searched along single muscle fibres to discover whether this procedure, which is experimentally better suited to the purpose, produces a change in the chronaxie of the muscle fibre. In all the measurements which have been made, there is no change in the chronaxie until the very tips of the fibres are reached, although there is usually a tapering off in

the fibre diameter. Results of stimulation at the tip can be divided into three groups. In one group of fibres, no change in the chronaxie is observable. In a second group, which is numerically the largest, it has not been possible to produce any response by excitation at the tip even though the stimuli are increased a hundredfold above the rheobase for stimulation in the body of the fibre. In a third group of muscle fibres the response to stimulation at the tip shows the curious long latent period reported by Moore and Brücke. This latent period is of the order of seconds and may well be due, as the above authors (following Hintner [1930]) believe, to a decrement in transmission of excitation. The threshold for this response is very irregular, changing as much as 50 p.c. Because of this, it is impossible to be certain of the chronaxie measurements. However, the chronaxie apparently increases, sometimes double or more.

TABLE II. Variation of chronaxie in different muscle fibres when locus of stimulation is changed.

Fibre	Locus of cathode	Threshold potentiometer scale	Chronaxie μF	Remarks
I	Body of fibre	140 ($V=10$)	0·062–0·065	Small fibre
	Tip of fibre	185	0·058–0·062	
II	Body	122 ($V=6·5$)	0·068–0·069	Fibre diameter about 3 times that of I
	Tip	($V=45$) (No response at full strength)		
III	Body	130 ($V=10$)	0·06–0·062	Large fibre
	Tip	370 ($V=40$)	0·15	Latent period of about 2 sec.

In Table II are shown sample results of measurements from each of these three different groups, made on three fibres from one preparation. No change in chronaxie was observed in the first fibre. The second fibre could not be stimulated at the tip, while there is an increase in the chronaxie of the third fibre from 0·06μF (0·24σ) to 0·15μF (0·6 σ), accompanied by a latent period of approximately 2 sec.

These results show therefore that Moore and Brücke's assumption, that the chronaxie of a single fibre depends upon the diameter of the fibre at the locus of stimulation, may be valid for some muscle fibres. However, this assumption apparently cannot be used to explain the heterochronism found in the gross preparation, since the extremely long latent period exhibited in the response of these fibres to stimulation at the tip is not to be found in the measurements of Rushton [1931]. It is hardly necessary to remark that the explanation offered by Moore and Brücke does not apply at all to the present work.

A second type of experiment for testing this assumption of Moore and Brücke consists in determining the chronaxies of different fibres of various diameters in the same preparation. The diameters of the fibres in the retrolingual membrane vary from about 15μ to 60μ or more. No relation has been found, however, between fibre diameter and chronaxie, such as is postulated by Moore and Brücke. On the contrary, it has been often found that large fibres may have chronaxies as much as four

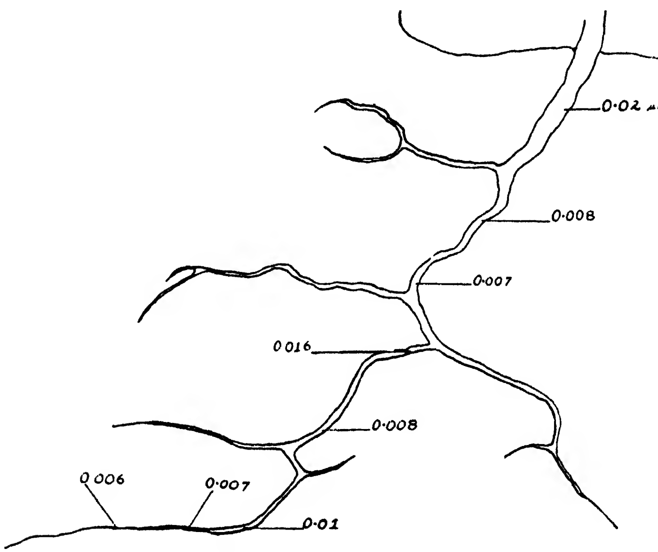

Fig. 6. Diagram of a nerve branch containing at least two fibres. This branch has been stimulated with a capillary electrode located at the various points to which reference lines have been drawn. Throughout the stimulation the response of one muscle fibre about 4–5 mm. distant was observed so that the chronaxies obtained along the branch represent those of a single nerve fibre. These values are shown in the figure and indicate that the chronaxie of a nerve fibre is not a fixed value but varies in a manner discussed in the text.

times greater than the chronaxies of very small fibres. The attempt of Moore and Brücke to reconcile their chronaxiemetric results with the theory of isochronism therefore seems to me to be without adequate experimental justification.

A further point to be discussed is in relation to the extremely small chronaxies which I have found for the nerve fibres. A possible explanation for this was pointed out to me by Prof. E. D. Adrian while he was a visitor at the Johnson Foundation. This explanation assumes that the small chronaxies are due to the fact that the single nerve fibres are being

stimulated in the membrane, where they are relatively free from connective tissue. It is in line with the results of Bishop [1928], who found that the chronaxie of a nerve trunk decreases when the sheath is removed. In agreement with this explanation I find that the chronaxie of a hypoglossal nerve fibre is approximately doubled when it is stimulated at one of the branches which is sent out by the nerve trunk into the tongue.

This change of chronaxie along the nerve fibre which seems to be related to the amount of inert connective tissue surrounding the fibre is perhaps illustrated more strikingly in the following experiment. The nerve branch which is shown diagrammatically in Fig. 6 has been stimulated at various locations indicated by reference lines in the figure. Near each line is also given the chronaxie found at this point. This nerve branch probably contained at least two fibres. However, all the measurements shown in this illustration have been obtained while observing throughout the response of one muscle fibre, and therefore represent the change in chronaxie which is found at various points along a single fibre. The muscle fibre itself had a chronaxie from 0·04μF (0·16σ) to 0·056μF (0·22σ), depending upon the proximity of the capillary electrode. It was 4–5 mm. distant from the region of the nerve stimulation. The nerve measurements were made with the electrode always as close as possible to the nerve. They show that in the thicker portion of the branch the nerve fibre had a chronaxie of 0·02μF (0·08σ), while at the very thin region the chronaxie fell to 0·006μF (0·024σ).

Discussion.

The results described in this paper show that the "law of isochronism" is not valid for the retrolingual preparation. Since there is no reason to assume that this tissue is unique, we must arrive at the conclusion that the law holds no better for any other nerve-muscle complex. They also force, however, a re-examination of the meaning and value of the concept of chronaxie. A brief review of this nature will be made in the present section.

Chronaxie, as a unit of time, seemed to Lapicque [1926] to possess many advantages. It was a quantity easily determined and formed the basis of a convenient description of the strength-duration curve. The concept of chronaxie led him to the various theoretical conclusions embodied in the law of isochronism and also to the idea that the chronaxie reflects closely such properties as velocity of propagation in nerve and in muscle.

It has become more evident, however, that the experimentally determined value of chronaxie depends on a number of factors. Davis [1923] showed that the chronaxie of muscle depends upon electrode size. His results were confirmed by Watts [1924]. In the present work this dependence upon electrode size has again been confirmed for muscle and has also been found for nerve.

Lapicque [1931 *b*] has recently suggested that the experimentally measured quantity also changes with a change in the type of surrounding medium. This seems a very reasonable suggestion and is supported by his measurements. He assumes, however, that chronaxies determined when a preparation is covered with Ringer's solution do not represent "true" chronaxie, while measurements made with a preparation which is only moistened with such a solution indicate "true" chronaxie. This classification seems to me arbitrary and one which does nothing to advance the merits of chronaxie as a theoretical concept.

Results given in this paper indicate that the chronaxie of nerve fibres, besides being determined by electrode size, is also largely influenced by the amount of connective or inert tissue around the nerve. These results present further difficulties to the concept of chronaxie, since they show very clearly that the chronaxie cannot be considered as a unit of measurement independent of experimental conditions and of structural modifications in the tissue.

An examination of its definition discloses the probable causes for these changes which the value of chronaxie undergoes. The strength-duration curve is measured in terms of the liminal voltage or current which will excite when applied for any given duration. The chronaxie, τ, is then expressed as the duration at which V, the stimulus strength, equals $2R$; where R is the rheobasic strength. However, as Lapicque points out [1926, p. 234], it is not the current strength but the current density which is the effective measure of an electrical stimulus. The current density, ρ, is some function, $f(V)$, of the strength, V. This function depends upon all the variables discussed so far, namely, the geometry of the electrode system, and the specific conductivity of the solution and of the various inert and excitable tissues which may be present. Conceivably, the area over which the current is spread upon the tissue is also a factor in determining a threshold stimulus. In such a case, the relative sizes of tissue and electrode will likewise influence the strength-duration curve. Bishop [1928] has discussed various other factors which may affect the precise form of the function $f(V)$. It can, therefore, be readily appreciated that the strength-duration curve can

change its form whenever $f(V)$ is changed, and the chronaxie, or τ, at $V = 2R$ will also assume different values. It is therefore evident that it is impossible to speak of any single and unique chronaxie for a given muscle or nerve. Consequently the chronaxie can no longer be accepted as an adequate unit in the description of strength-duration curves or as a basis for theoretical discussions regarding the nature and course of the excitation process.

We are now in a position to inquire into the possibility of bringing together more closely the results obtained in the study of the single fibre nerve-muscle complex with those of Lucas and of Rushton on the gross preparation. With the presentation of Rushton's latest analysis [1932, in press], it has become quite clear that the complex in the gross preparation is composed of two elements. These are the excitable substances in nerve and muscle. The results just presented show clearly the same distinction between the excitable substances in nerve and muscle fibres. The time relations which are found for these substances are, however, different in these two types of nerve-muscle preparations. For the muscle fibres this difference, as Rushton has already assumed, is due to the effect of electrode size found by Davis [1923]. Up to the present work, however, it has been assumed that the excitation time of nerve fibres is little affected by electrode size. Indeed, the γ-curve definitely has [Rushton, 1932*c*] an excitation time of the order of magnitude found for the nerve trunk. When single nerve fibres relatively free from connective tissue are stimulated however, it is found that the excitation time also depends on electrode size, and becomes very small with small electrodes. It is exceedingly likely, therefore, that the γ-curve of gross preparations is a counterpart of the curve obtained for single fibres, but with the time relations, owing to the use of large electrodes, altered in the manner which has been discussed in the preceding pages.

CONCLUSIONS.

The measurements on the strength-duration relationship in the single fibre nerve-muscle complex yield direct evidence that:

1. Lapicque's law of isochronism is not valid, at least for the system in the retrolingual membrane.

2. The view held by Lucas and by Rushton which assumes that different excitable substances are present in nerve and in muscle is in agreement with the results obtained from a study of the single fibre complex.

These two conclusions find experimental justification from the following lines of evidence:

(*a*) The voltage-capacity curves of muscle and nerve fibres are different in shape.

(*b*) This difference can be manifested by the production of compound curves, which can be analysed directly into the two components obtained from stimulation of nerve and of muscle.

(*c*) The chronaxie of nerve fibres is much smaller than is the chronaxie of muscle fibres.

(*d*) Curarization does not affect the chronaxie or the strength-capacity curve of muscle fibres.

The present work also yields experimental evidence that:

3. The compound curves obtained in the gross preparations are strictly homologous with those obtained for single fibres. The difference in time relations in the two experimental procedures is brought about by two factors:

(*a*) the excitation time of muscle fibres is increased with increased electrode size, and

(*b*) the same effect exists, to a lesser degree, in nerve fibres.

4. The chronaxie of nerve fibres decreases with a decrease in the connective tissue sheath. This conclusion is based on the fact that stimulation of the nerve fibre in the trunk or in a branch produces an increased chronaxie.

5. Because the chronaxies of both muscle and nerve are dependent upon the electrode size while that of nerve also varies with the amount of connective tissue sheath, it seems legitimate to question the further usefulness or significance of Lapicque's concept of "chronaxie." It no longer appears to have the feature of being a simplifying and generally applicable unit of time in the description of the strength-duration curve.

6. Finally, it is concluded that the explanation offered by Moore and Brücke [1931] for the heterochronism which has been discovered in various nerve-muscle preparations does not seem to have any experimental justification. Their explanation assumes, from analogy with the results for nerve [Lapicque, 1926], that a large chronaxie in muscle is due to stimulation of the individual muscle fibres in a locus where the fibres have a small diameter. No such effect of fibre diameter upon chronaxie has been found, except in the case where the response to direct stimulation of the tip exhibits a latent period of the order of seconds.

Summary.

The present investigation is concerned with the determination of the relation between the intensity of an electrical stimulus and the minimum duration for which it must be applied in order to stimulate single nerve and muscle fibres. It was undertaken with the purpose of testing the merits of the conflicting views of Lapicque [1926] and of Lucas [1907] and Rushton [1930, 1931, 1932 *a*]. The preparation used for this purpose has been the single fibre nerve-muscle complex of the retrolingual membrane of the frog *R. pipiens*.

The conclusions reached from the results of this investigation are stated in the previous section.

I wish to thank Prof. D. W. Bronk, director of the Johnson Foundation, for his kindness and help during the course of this work. I am also greatly indebted to Dr W. A. H. Rushton for the stimulation and aid which he generously bestowed during his residence at the Johnson Foundation.

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THE EFFECT OF FATIGUE ON END-PLATE DELAY.

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Introduction.

The classical experiment to demonstrate the nature of the failure of indirect excitation in fatigue is to stimulate the nerve of a muscle-nerve preparation until the muscle ceases to give a contraction, and then to show that a response may still be evoked by direct stimulation. From this result it is argued that, since the fatigue is not due to any change in the nerve (which can be shown to be conducting normally), nor to any obvious alteration in the muscle itself, it is necessary to postulate an intermediary tissue, functionally distinct from nerve and muscle, which has the property of transforming the energy of the nerve impulse into a stimulus for the muscle, and which is particularly susceptible to fatigue.

The conception of the separateness of the junctional tissue derives considerable support from the well-known experiments of Claude Bernard on curarization and from the detailed investigation of Langley [1907], who put forward the hypothesis that the intermediary is specially sensitive to such influences as nicotine and curare. Further evidence was supplied by Keith Lucas [1907] from experiments on the time relations of excitability of frogs' sartorius muscles. Lucas found that, if the point of stimulation is in the region of the muscle where the nerve enters, the strength-duration curve generally exhibited one or two breaks which he explained as being the result of stimulating at a point where the excitatory process could influence both nerve and muscle: in that part of the muscle where no nerve endings could be seen, a smooth curve was always obtained. The slowest curve he regarded as being derived from excitation of the muscle; the middle one, which was abolished by curare, he attributed to nerve; and the fastest of all, which was not affected by small doses of curare, he tentatively suggested might be regarded as

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characteristic of the neuro-muscular junction. This interpretation has not, however, been generally accepted.

It has been known for a long time that, in the indirect stimulation of muscle, the time which elapses between the moment of stimulating the nerve and the appearance of the muscle-action potential is longer than can be accounted for by conduction in the nerve, unless, as Lapicque and others have suggested, the rate of conduction in the terminal branches is slower than in the nerve trunk where it can be measured. As these branches are generally smaller than the fibres in the trunk, it is quite probable that they conduct more slowly, though this does not necessarily follow, since the experiments of Gasser and Erlanger [1926], which might seem to suggest this conclusion, were concerned with the rates of conduction in fibres of different function.

This delay in the transmission of excitation from nerve to muscle has been investigated by Forbes, Ray and Griffith [1923]. They found that if two stimuli are sent in to a motor nerve with only about 4σ between them, the second stimulus sets up an impulse which is delayed a relatively long time at the myoneural junction. The interpretation of these results which is given by Forbes, Ray and Griffith is that, at the junction of the nerve with the muscle, the excitatory condition due to the second stimulus may persist until the threshold of the muscle, raised by the first response, has fallen sufficiently to allow the second impulse to take effect. This explanation involves the assumption that the effective portion of the nerve impulse lasts as long as 5σ, a duration considerably greater than that usually accepted for frog's nerve at 20° C. Alternatively, the delay may be attributed to a separate substance which is able to remain in the excitatory state for a longer period than nerve. A means of avoiding both these assumptions will be suggested later.

The delay observed even in the transmission of a single impulse was attributed to a separate substance by Wieser [1915], who investigated the effect upon it of fatigue. Wieser's measurements were of the latent period between the stimulation of a motor nerve and the appearance of the muscle-action potential. He found that after a nerve-muscle preparation had been fatigued by tetanization, the latent period was often increased by as much as 100 p.c. Samojlov [1925] improved upon Wieser's technique by placing one lead of a string galvanometer on the nerve of a sciatic-gastrocnemius preparation at the point where it enters the muscle, and the other lead on the tendon of the muscle. In this way he avoided the possibility of error due to changes in the rate

of conduction in the nerve. He measured the effect of changes of temperature upon the delay, and from his results calculated the very high Q_{10} of 2·0 to 2·6, a value much greater than that obtained by Fulton, in whose book [Fulton, 1926] the whole subject is treated in detail. Samojlov deduced from his figures that transmission in the motor end-plate is "more chemical" than in either nerve or muscle. The temperature coefficients of biological systems cannot, however, be considered as affording very exact evidence as to the nature of those systems.

METHOD.

The purpose for which this work was undertaken was to discover if curare had any effect on the delay as measured by Samojlov. The

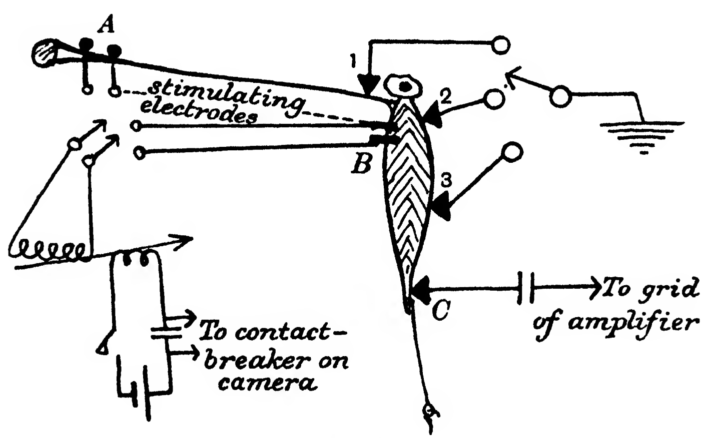

Fig. 1. Arrangement of electrodes on sciatic-gastrocnemius preparation.

arrangement of electrodes was as in Fig. 1. Since the string galvanometer cannot give accurate records of currents of short duration, a thermionic amplifier and Matthews' oscillograph were substituted. These were worked with a specially constructed rotating mirror camera of the type described by Matthews [1929]. With this it was possible to take records at any speed up to 5 m. per sec. Calibration photographs were taken at the beginning and end of each experiment. Stimuli were given by a coreless induction coil, in the primary circuit of which was a contact breaker operated by a cam on the driving shaft of the camera. Just maximal shocks were used throughout. The number of stimuli given to the tissue per second varied with the camera speed. In the

case of Figs. 4 and 5 stimulation was at the rate of six per second, in Figs. 6 and 7 at two per second. It was not found that the rate of stimulation affected the results. The contraction of the muscle was isometric. All the preparations were made from *Rana temporaria* in the winter, and the temperature was kept constant at about 15° C. during each experiment. It did not vary by more than two degrees from experiment to experiment.

The first preparation to be used was the ordinary sciatic-gastrocnemius. It was soon clear, however, that no accurate or reproducible results could be obtained with this muscle, owing to the complex character of the action potential, and to great variability in its point

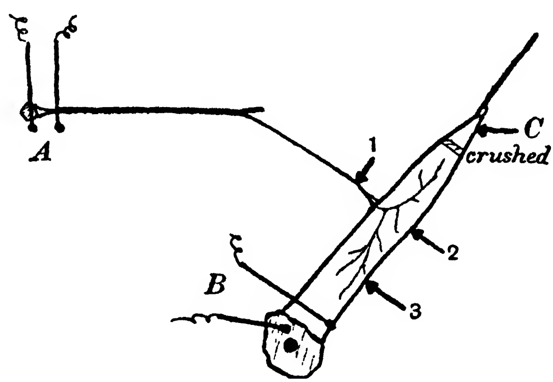

Fig. 2. Arrangement of electrodes on sartorius preparation.

of origin. This result was perhaps to be expected from the anatomical arrangement of the fibres. Generally a point just above the belly of the muscle was the first to become active, but often, as the muscle became fatigued, the point would shift, sometimes to such an extent that the sense of the action potential would be reversed, indicating that the first point to become active was nearer the grid electrode on the tendon. Nearly always a polyphasic action potential appeared in the course of fatigue. It was therefore decided to use the sartorius muscle and its nerve (Fig. 2). Electrodes 1, 2 and 3 and the stimulating electrodes *A* and *B* were made of silver wire 1·25 mm. in diameter coated electrolytically with silver chloride and fixed to ball and socket holders. The grid electrode was of the wick type. Usually the tendon end of the muscle was crushed in order to obtain monophasic action potentials and to increase their amplitude when the leading-off electrodes were close together. It was

not found that this treatment was detrimental to the tissue. The muscle was suspended in air and frequently irrigated.

When the preparation was first set up after equilibration in oxygenated Ringer's solution for 1 hour, a photograph (Fig. 3 *a*), was quickly

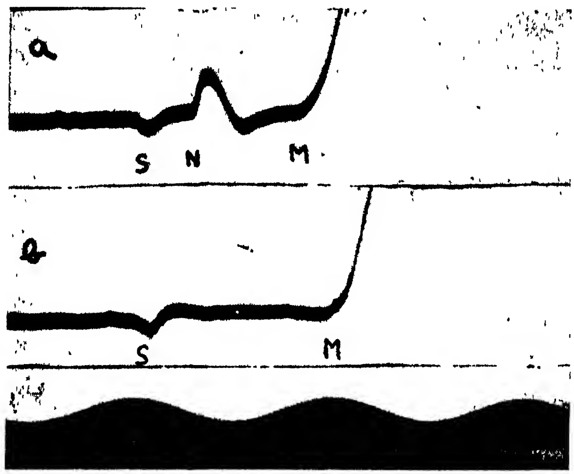

Fig. 3. Action potentials from fresh preparation. Time marker gives 180 d.v./s. *a*, leads 1 *G*; *b*, leads 2 *G*. Stimulation at *A* (Fig. 2).

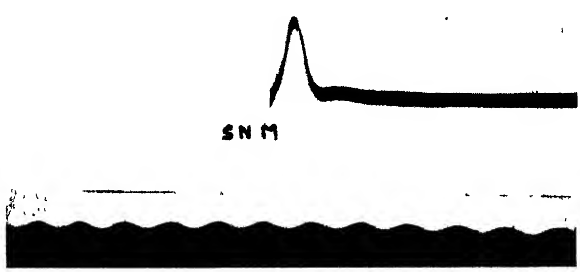

Fig. 4. Action potentials from fresh preparation, using lower amplification. Time marker gives 180 d.v./s. Leads 1 *G*. Stimulation at *A* (Fig. 2).

taken with leads 1 *G* (Fig. 2), closely followed (Fig. 3 *b*) by one with leads 2 *G*. Fig. 4 was taken at a lower speed and with lower amplification. The muscle-action potential, *M*, is simple in character and is preceded by the nerve-action potential, *N*, and the stimulus escape, *S*. It will be seen that in Fig. 3 *a* the distance *S–M* is slightly less than in Fig. 3 *b*. The probable reason for this is that in the sartorius preparation the

electrode 2 is on the side of the muscle opposite to the point of entry of the nerve: the fibres under it will receive the nervous stimulus slightly later than those connected by the nerve itself to electrode 1. In the gastrocnemius preparation, on the other hand, the point at which the nerve comes into contact with the muscle is not that at which the muscle is first excited. This explains Samojlov's results in which records obtained with either leads 2 *G* or 3 *G* (Fig. 1) gave a shorter duration *S–M* than those obtained with leads 1 *G*.

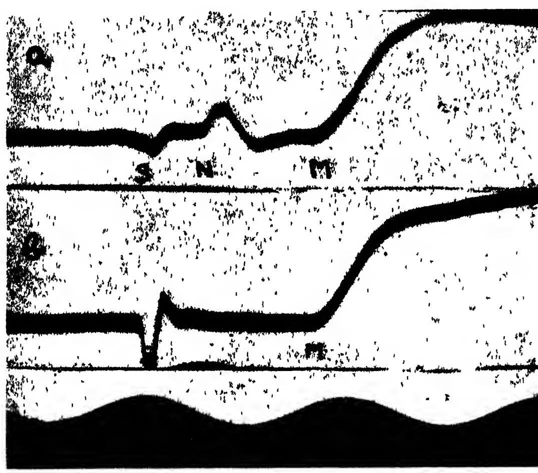

Fig. 5. Same preparation as Fig. 3 after stimulating for 3 min. at six stimuli per second. Amplification and time marker as in Fig. 3. *a*, leads 1 *G*; *b*, leads 2 *G*. Stimulation at *A* (Fig. 2).

It was then intended, as stated, to poison the muscle with curare; in the preliminary experiments, however, in which the apparatus was being adjusted and the preparation was unavoidably becoming fatigued, observation did not seem to verify Wieser's results. Attention was accordingly directed to this problem. The preparation was fatigued by indirect stimulation at the rate of six stimuli per second, the changes in the action potentials being meanwhile observed on the viewing screen. When the muscle action potential as seen on the screen had shrunk to one-tenth of its original amplitude, two photographs (Fig. 5) were taken as before. At this stage the contraction of the muscle was barely perceptible.

RESULTS.

From a comparison of the second pair of photographs with those from the preparation when fresh, it is evident that there is no detectable increase in the distance *N–M* as measured from the beginning of the nerve-action potential to the beginning of the muscle-action potential. This result was obtained on all sartorius preparations.

Further experiments with the gastrocnemius showed that results similar to Wieser's could be obtained with this muscle when the electrodes were suitably placed. With electrodes 1 *G* or 2 *G* (Fig. 1), the delay

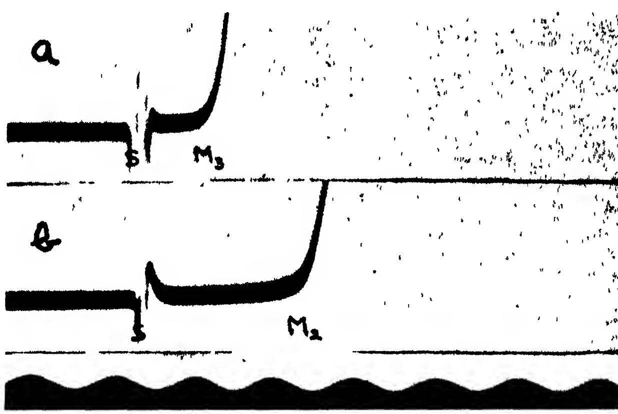

Fig. 6. Muscle action potentials from fresh preparation. Time marker gives 180 d.v./s. *a*, leads 3 *G*; *b*, leads 2 *G*. Stimulation at *B* (Fig. 2). Conduction rate 1·5 m. per sec.

in the fresh preparation was found to be greater than with the corresponding leads on the sartorius preparation. Further, this delay increased in fatigue by as much as 60 p.c. On the other hand, in some preparations, leading off from lead 3 quite close to lead *G* gave results similar to those obtained with the sartorius. It was suspected that these inconsistencies might be traced to changes in the rate of conduction in the muscle itself, for the nearer the proximal lead was to the point of first activity of the muscle, the less was the discrepancy between gastrocnemius and sartorius. The difficulty with gastrocnemius was, as has been mentioned, that the point of first activity often shifted in the course of an experiment.

A sartorius preparation was then stimulated directly through the leads *B* (Fig. 2), and a pair of photographs (Fig. 6 *a* and *b*) taken with

leads 3 G and 2 G respectively. In this way it was possible to avoid any error due to the spread of the stimulating current. The rate of conduction from lead 3 to lead 2 was measured by subtracting the distance S–M 3 from S–M 2 (Fig. 6). The muscle was slowly fatigued and photographs (Fig. 7) were taken when the action potentials were one-tenth their original size. In the case of the preparation from which Figs. 6 and 7 were taken, the rate of conduction when fresh was 1·5 m. per sec., and dropped in fatigue to 0·9 m. per sec. This agrees well with Wieser's experiments, in which the delay, which he thought due to the end-plate, was about 60 p.c. greater in the fatigued preparation than in the fresh one.

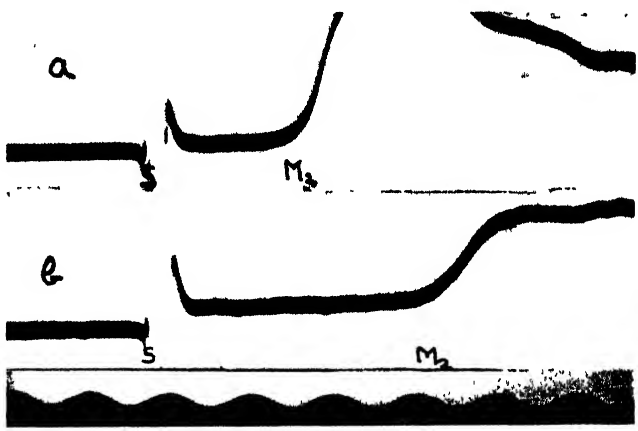

Fig. 7. Same preparation as Fig. 6 after stimulating for 5 min. at two stimuli per second. Amplification twice that in Fig. 6. Time marker gives 180 d.v./s. a, leads 3 G; b, leads 2 G. Stimulation at B (Fig. 2). Conduction rate 0·9 m. per sec.

The experiments on indirect stimulation were repeated with varying positions of lead 2 (Fig. 2). It was found that the delay increased as the lead was moved farther toward the pelvis away from the region of the muscle where nerve endings are found, and that the longer the delay was, the more it was affected by fatigue.

There can be little doubt that the delay measured by Wieser, Samojlov and others was the sum of the true delay and the muscle conduction time, though Wieser, at least, thought that he had made adequate control experiments. The changes observed in fatigue must similarly have been changes in the muscle itself.

Discussion.

The results of Forbes, Ray and Griffith in connection with the long delay experienced by a second impulse given to a motor nerve may be easily explained if it be supposed that in their experiments also the muscle conduction time was included in measurements of the delay. As the second impulse would stimulate the muscle in its relative refractory period, the muscular impulse would be conducted more slowly to the leading-off electrodes, thus causing an apparent delay in the appearance of the muscle-action current.

The constancy of the end-plate delay in fatigue weakens the case for the existence of the hypothetical intermediary substance; further doubt is cast upon its reality by consideration of the actual value of the delay. From Fig. 4 *a* it is seen that the distance $N-M$ corresponds to a time of about 3σ, and this figure has been checked by observation of many preparations, both photographically and by measurement on the viewing screen. Now it is difficult in the sartorius-nerve preparation to bare the nerve right up to its point of entry into the muscle, so that in most cases the distance from lead 1 to the point inside the muscle where it may be presumed excitation occurs was generally about 1·5 cm. In the case of the preparation from which Fig. 4 was taken, the rate of conduction in the nerve was measured by leading off action potentials from two places, as for the muscle, and was found to be 15 m. per sec. The distance of 1·5 cm. between lead 1 and the point of action of the nerve would therefore be traversed in about $1{\cdot}0\sigma$; this time may be subtracted from the delay observed. The remainder, 2σ, may be divided into two parts: the first due to the latent period and conduction time of the muscle, and the second to some process yet to be accounted for. The amount due to conduction in the muscle must be small, since the increase in conduction time which has been shown to occur in fatigue would lead to the detection of a delay due to this source if it were greater than $0{\cdot}25\sigma$. Consideration of the latent period of the muscle raises the question as to what is the effective stimulus for the muscle. If, as Lapicque, for example, believes, the stimulus is merely the current due to the nerve-action potential change, it is unlikely that the moment at which the action current sets up a muscular impulse will occur sooner than the end of the rising phase of the action potential. In Fig. 4 the action potential of the nerve is diphasic, and the rising phase is curtailed for this reason: monophasic recording from the same nerve showed that the actual duration of the rising phase was approximately $1{\cdot}2\sigma$. This time may therefore be sub-

tracted from the delay, leaving 0·8σ unaccounted for. Apart from the possibility that there is a delay associated with a separate intermediate substance—a possibility not ruled out by these experiments—there are two alternative explanations of the small delay remaining. Either a slower conduction rate may be postulated for the terminal branches [Fulton, 1926], or it may be that the utilization time by the muscle of the nerve-action current is longer than 1·2σ. There is nothing to show which of these explanations is the true one, nor are they mutually incompatible. The question is discussed in some detail by Forbes, Ray and Griffith [1923, pp. 603–9].

The conclusion to be drawn from these results is that, if there is an intermediary substance, any function which it may be shown to have must be such as can be performed in a very short space of time. This renders unlikely the existence of a "receptive substance," and it would seem more satisfactory, therefore, to attempt to explain fatigue on the basis of changes which are known to occur in muscle, rather than on the assumption of changes which cannot be observed in a hypothetical substance.

The effect of curare, both upon indirect excitation as measured in these experiments, and upon the characteristics of the muscle response evoked by direct stimulation, should throw light on this problem.

Summary.

1. Measurements have been made of the delay in the transmission of activity from nerve to muscle by leading off the electrical changes in both to an amplifier and Matthews oscillograph.

2. Fatigue causes no increase in the delay when a sartorius-nerve preparation is used.

3. Observation of the changes in muscle conduction rate resulting from fatigue indicate that the changes hitherto attributed to an intermediary substance were changes in the characteristics of the muscle.

4. Values for the delay derived from experiments on the sciatic-gastrocnemius preparation are too high, being the sum of the true delay and the conduction time in the muscle.

5. In winter specimens of *Rana temporaria* at 15° C., the delay is never more than 2·0σ. Under these conditions the rate of conduction in the nerve is 15 m. per sec.

6. It is suggested that there is no need to postulate either a slower conduction rate in the terminal nerve fibres or the existence of a separate

substance in order to account for the delay, which is less than the duration of the nerve action potential.

It is a pleasure to record my deep gratitude to Mr Matthews for his advice and instruction and to Dr Rushton for his helpful criticism.

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THE REGULATION OF THE PYLORIC SPHINCTER.

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Considerable discussion has taken place as to the mechanism regulating the opening and closing of the pyloric sphincter since Cannon in 1907 enunciated his theory of the acid and alkali control. Unfortunately, as Alvarez [1928 *a*] points out, commentators have not infrequently quoted incorrectly and, further, have failed to note that Cannon had seen defects in his theory, as in certain conditions it did not explain all the facts.

An excellent summary of the literature on the control of the pylorus is given by Alvarez. In most of the investigations described, attention has been directed to the consistency or reaction of the foodstuff, and little or no regard has been paid to the analysis of the movements of the pyloric antrum and sphincter. Wheelon and Thomas [1920, 1922], however, have studied the relation between the sphincter and antral contractions in dogs by means of an enterograph introduced into the antrum. These observers show that the pyloric sphincter has cycles of rhythmic motility and that these cycles are coincident and of the same duration and rhythm as those of the antrum proper. "A phase of inhibition (maximal relaxation) manifests itself during the height of antral contraction. Following this, and while the antrum is finishing its contraction, the sphincter begins its positive phase and reaches the height of its contraction when the antrum is rapidly relaxing or has relaxed. Following this, the sphincter relaxes." They further suggest that the sphincter may act not only in relation to the antrum to aid in the propulsion of material from the stomach, but also as a barrier to the regression of chyme during the presence of a positive phase of the duodenum.

The co-ordination which exists between the movements of the antrum and the pyloric sphincter and the part played by the sphincter in regulating the passage of fluid may be studied by using the preparation described by McCrea and McSwiney [1926]. It has been possible to

separate the antrum from the body of the stomach without interference with the nerve or blood supply and to record simultaneously the contractions of the antrum and the amount of fluid flowing through the sphincter. The method has distinct advantages, as there is no disturbance of the pyloric sphincter.

Method.

Dogs have been used in these experiments. The animal was first anæsthetized with ether, sustained anæsthesia being obtained by intravenous chloralose (0·075 g. per kg.). A second injection was occasionally necessary some 2–4 hours later. The abdomen was opened through a mid-line incision, and the body of the stomach separated from the pyloric antrum. The distal end of the body was then closed by a running Czerny-Lembert suture. A wide-bore cannula was inserted and tied into the antrum. Alternatively, a ligature may be passed through the greater and lesser omentum close to the curvatures of the stomach at the incisura, and tied tightly round the junction of the antrum and the body; a small opening is made into the antrum near to the point of ligature, and a cannula inserted and securely tied.

A method suggested by Babkin [1931] was used in several of the earlier experiments. In the region of the incisura a longitudinal incision 1 in. long was made through the seromuscular coat to the mucosa midway between the greater and lesser curvatures, and a tube of mucous membrane was separated from the seromuscular coat. A cannula was inserted into the tube which was then tied off.

The duodenum was divided transversely 1½ in. from the pylorus. A wide cannula was tied into the proximal end, 1 in. distal to the pylorus. Rubber tubing connected the cannula with a U-tube manometer. The fluid, escaping from the antrum, flowed into one limb of the manometer, and was recorded on the kymograph by the movement of the waterfloat; the level of the fluid in the manometer may be lowered after a series of contractions to its resting level.

An interval of approximately 30–60 min. was allowed to elapse after the operation; the cannula in the antrum was then attached to the tube leading from a reservoir supported 2 ft. above the level of the antrum. A valve was placed at the lower end of the tube, and this allowed fluid to flow into the antrum only during the relaxation phase. A tube, attached to a tambour for recording antral contractions, was connected to the inflow tube below the valve.

The tap of the reservoir was opened, and the rate of inflow was varied

by inserting capillary tubes of different calibres into the rubber cork of the reservoir. The reservoir is placed at a height above the animal to ensure "diastolic" filling. The pressure was never sufficient to open the sphincter, which is able to resist comparatively high pressures. The pressure of the fluid does not therefore determine the rate of escape of fluid from the antrum, but merely ensures adequate filling.

EXPERIMENTAL RESULTS.

Simultaneous records of the contraction of the antrum and the amount of fluid flowing through the sphincter were first obtained using 0·9 p.c. sodium chloride solution as the inflow fluid. Slight movements are observed even before fluid fills the antrum, but well-marked contractions do not occur until the antrum is distended [Ducchesi, 1913]. The amplitude of contractions varied in different animals, but the height of the contraction wave was remarkably constant in the same animal for any given rate of inflow. The height of contraction was found to vary with the inflow, and, if the rate of inflow was increased, the amplitude of contraction increased. The form of the antral contraction was observed to be constant, and consisted of an even ascent and descent of the curve, except for a concavity on the rising limb which marks the opening of the pyloric sphincter. Small superimposed respiratory waves are usually present. A large contraction occurring in a series of regular waves was generally preceded by a longer period of diastole. The frequency of contraction remained remarkably constant throughout the experiment, and little or no change was observed on altering the inflow.

The saline solution was expelled from the antrum through the sphincter in spurts or jets which synchronized with the antral contractions. The outflow of fluid from the antrum was recorded, and, as fluid only escaped during the antral systole, a step-like tracing was obtained. If fluid enters the antrum too rapidly, or if, owing to the bad condition of the animal, the sphincter is not able to hold back the fluid, the tracing illustrating the outflow becomes a continuous rising line. The state of the pyloric sphincter is therefore indicated by the type of outflow curve.

It has previously been observed that the height of the antral contraction varies with diastolic filling. In the same way, the amount of saline solution expelled with each contraction varies with the inflow. With a constant rate of inflow, the amounts expelled at each contraction vary slightly from one another, but increase in inflow causes a greater antral contraction with a corresponding increase of outflow.

TABLE I.

Inflow	Height of contractions (antrum) cm.	Frequency of contractions (antrum) per 3 min.	Average outflow per contraction c.c.
Small	2·3–3·2	12	4·8
Medium	2·4–4	14	7·7
Large	2·5–5·5	16	8·9

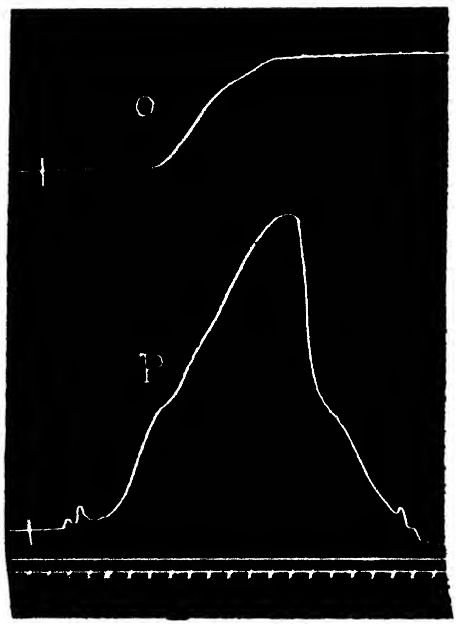

Fig. 1. Tracing to show relation between (*P*) contraction of pyloric antrum and (*O*) outflow of saline through the pyloric sphincter. Time interval 1 sec.

The size of antral contraction is not, however, the only factor regulating the outflow, as not infrequently a larger amount of fluid is expelled with a small contraction than with a large contraction. It would appear, therefore, that the amount of outflow with a constant inflow depends on (*a*) the contraction of the pyloric antrum, and (*b*) the relaxation of the pyloric sphincter. It is clear that the extent and duration of the relaxation period of the sphincter must be the principal factor determining the outflow.

Tracings illustrating the relation between the movements of the antrum and the pyloric sphincter (Fig. 1) have been obtained. The time

intervals will, of course, vary in each experiment according to the frequency of contraction and other factors.

In Table II a series of measurements obtained with saline is recorded. It will be observed that the outflow commenced some 2–4 sec. after the

TABLE II. Saline, constant inflow.

	Height of contraction (antrum) cm.	Amount of outflow c.c.	Time of outflow sec.	Contraction (antrum) Ascending sec.	Contraction (antrum) Descending sec.	Interval between contraction and outflow sec.
Dog 1	6·3	4	5	8·5	7	2·5
	6·3	4·4	6	10	7·5	3·5
Dog 2	3·7	1·2	5	9	8	3·5
	4·6	2·4	8	12	6	3
Dog 3	3·2	3	3·5	4	4·5	3
	2·1	3	2	4·25	6	4
Dog 4	4·5	2·8	4	8·75	5	3·75
	3·7	2·8	4	7·75	4	3

onset of the contraction of the pyloric antrum. The outflow usually lasted for some 4–6 sec. and ceased before the antrum relaxed. It may, therefore, be stated that the pyloric sphincter relaxes for a period of approximately 4–6 sec. during the period of antral contraction. It must be emphasized that in all our experiments the sphincter remained contracted except for short periods of the antral cycle. The measurements given in Table II are not strictly accurate, as a lag occurs between the contraction of the antrum and the reception of fluid in the manometer.

A series of experiments has been made on the effects of various fluids upon the activity of the sphincter pylori. It is usually stated that fats leave the stomach at a slower rate than proteins, and again that solid and semi-solid foods empty less quickly than liquid. Considerable variation in the rate of outflow of liquids from the stomach is further described. Acid fluids empty at a slower rate than neutral solutions, and alkaline solutions empty at a rate which is between that of acids and neutral solutions [Alvarez, 1928 *b*].

To determine whether acid, alkali, salt, organic acid or viscous solutions influence the state of the pyloric sphincter, the passage of the following fluids through the pyloric antrum was investigated.

I. Sodium chloride, 0·9 p.c.; sodium chloride, 25 p.c.; distilled water; glucose, 25 p.c.; hydrochloric acid, 0·18 p.c. in normal saline; sodium bicarbonate, 0·25 p.c. in normal saline.

II. Olive oil; gum acacia, 10 p.c. and 25 p.c. in normal saline.

III. Organic acids, 2 p.c. solutions; lactic, malic, succinic, tartaric and caproic acids.

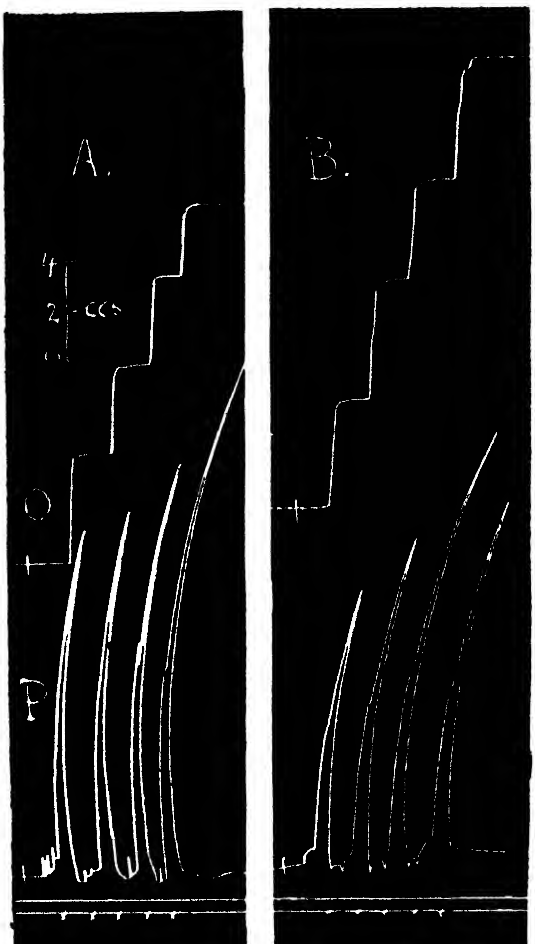

Fig. 2. Tracing to show relation between (*P*) contraction of pyloric antrum and (*O*) outflow of fluid through the pyloric sphincter. *A*, saline; *B*, alkali; *C*, acid. Time interval 30 sec.

The contractions of the pyloric antrum and the outflow of fluid through the sphincter were first recorded, using 0·9 p.c. sodium chloride solution. At the end of this initial experiment the inflow fluid was

changed over, and comparable tracings were taken with one of the test solutions. Each preparation was used to test three or four solutions.

TABLE III.

Fluid	Height of contraction (antrum) cm.	Amount of outflow c.c.	Time of outflow sec.	Contraction (antrum) Ascending sec.	Contraction (antrum) Descending sec.	Interval between contraction and outflow sec.
Saline	6·3	4	5	8·5	7	2·5
Saline	6·3	4·4	6	10	7·5	3·5
Alkali	4·4	5	6	10	5	2
Alkali	5·7	5	6	13	7	4
Acid	5·5	4·8	6	12·5	6·5	4·5
Acid	4·1	6·4	7·5	12	5	4·5

Two readings are given for each fluid, the first set of readings being taken shortly after the start, and the second towards the end of the experiment. The inflow was constant throughout.

The results obtained with the fluids of Groups I, II, III were in no way different from those obtained with saline. Records and measurements made using saline, alkali and acid are given in Fig. 2 and Tables III and IV. The base line of the antral contractions remained remarkably constant, demonstrating that there was no appreciable change of tonus. When the inflow fluid is first changed over, the antral contractions and the outflow curve occasionally become irregular for a short interval. The preparation, however, soon settles down, and regular records are obtained.

TABLE IV.

Fluid	Average height of contraction (antrum) cm.	Frequency of contraction (antrum) per 3 min.	Average outflow c.c.
Saline	5·9	4	4·3
Saline	6·7	4	3·8
Alkali	7	5	3·1
Alkali	6·8	5	4·5
Acid	6·5	6	4·8
Acid	7·2	6	4·3

Two readings are also given in this table for comparison as in Table III. The inflow was constant.

The regular step-like character of the outflow showed that the sphincter remained closed between the systoles of the antrum. If the rates of inflow were increased, a greater amount of fluid, as has been previously pointed out, was ejected at each contraction. With high rates of inflow, the step-like character of the outflow curve was lost, indicating that the outflow continued, though usually at a diminished rate during

the diastole of the antrum. With viscid solutions, the outflow curve, as might be expected, was not as clean cut as for other solutions.

It must be emphasized that in no instance was the passage of fluid from the antrum to the intestine influenced by the type of solution. In some experiments, however, an increase in the frequency of antral contractions was recorded with the different inflow fluids.

Discussion.

By the use of the isolated pyloric antrum preparation with intact nerve and blood supply, as described in this paper, it is possible to demonstrate that the movements of the pyloric antrum and pyloric sphincter are related. Criticism may be advanced that by the introduction of a valve between the reservoir and pyloric antrum, the condition of the antrum becomes abnormal. In preliminary experiments the valve was omitted, and results similar in all details were obtained, but the amount of fluid ejected from the antrum with each contraction varied considerably. It will be realized that in the normal stomach as digestion proceeds, the stomach becomes more tubular and the prepyloric sphincter is definitely formed [Cathcart, 1911]. Indeed, with a barium meal it is possible to see the antrum full of barium apparently completely separated from the remainder of the stomach. The valve was therefore introduced to imitate the effects produced by the prepyloric sphincter.

We are able to confirm the previous observations of Wheelon and Thomas [1920, 1922] that the movements of the antrum and sphincter are related, but we disagree with the statement that while the antrum is finishing its contraction, the sphincter begins its positive phase and reaches the height of its contraction when the antrum is rapidly relaxing or is relaxed.

Our results show that the pyloric sphincter is normally contracted, as can be seen from the step-like form of the outflow record. Following the contraction of the antrum, the sphincter relaxes and returns to its state of contraction in some 5–6 sec. The relationship between the contraction and relaxation of the sphincter depends mainly on the rate of antral contraction. According, however, to Wheelon and Thomas, the sphincter is usually relaxed and only contracts for a period following the antral contraction to act as a barrier to the regression of chyme during the presence of a positive phase of the duodenum. It is extremely difficult to see how the stomach could hold fluid if the normal state of the sphincter were relaxation. The rhythmic contractions of the pyloric sphincter recorded by Wheelon and Thomas are, in our opinion, due

at least in part to the stretching of the muscle fibres by the insertion of a balloon. For in the animal, as in isolated preparations, stretching of the muscle fibres acts as a stimulus for rhythmic contractions.

It has also been possible to demonstrate that the different solutions employed have little or no influence upon the pyloric sphincter when allowed to flow into the antrum. It has been pointed out that acid solutions are stated to empty at a slower rate than neutral and alkaline solutions at a rate between acid and neutral solutions. Again, Carnot and Chassevant [1905] have shown that the rate of emptying of hyper- and hypotonic solutions of glucose increased as the solutions approached isotonicity. Magee and Reid [1931] and McSwiney and Spurrell

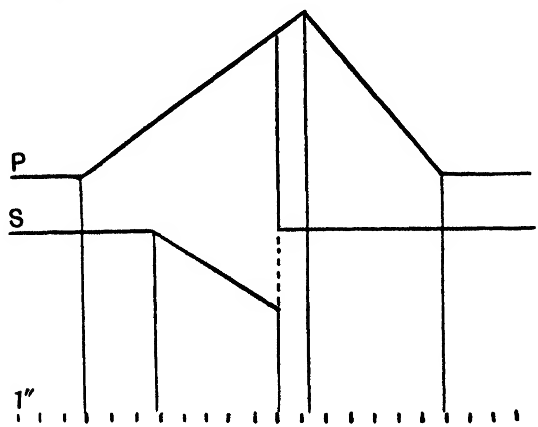

Fig. 3. Diagram to illustrate the relation between (P) contraction of the pyloric antrum, and (S) the relaxation of the pyloric sphincter.

[1932] have also suggested that osmotic pressure of the stomach contents may prove to be an important factor in regulating the emptying of the stomach. As the pyloric antrum and the sphincter are, according to our experiments, insensitive to factors which other authors state either increase or decrease the emptying time of the whole stomach, it follows that the body of the stomach must react by increase or decrease of tone according to the fluid or foodstuff it holds.

It is interesting to note the similarity which exists in some respects between the pyloric antrum and the right or left ventricle, as is evidenced by the rhythmicity of the antral contraction, the variation in height of contraction with diastolic filling, and the increase in outflow with corresponding increase in inflow. On the other hand, variation in inflow causes little or no change in the frequency of contraction.

Summary.

Experiments are described in this paper which demonstrate the relationship between the movements of the pyloric antrum and sphincter in regulating the passage of fluid from the stomach to the duodenum. The fluid was expelled from the antrum through the sphincter in spurts which synchronized with the antral contraction.

The sphincter, as judged by the outflow from the pyloric antrum, is normally contracted, and relaxes some 2–4 sec. after onset of the antral contraction for a period of some 6 sec. Acids, alkalis, hypo- and hypertonic solutions and solutions of different organic acids have little or no influence on the pyloric sphincter when allowed to flow into the antrum. The height of antral contraction and the outflow of the antral contents were found to vary with the inflow.

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FACTORS INFLUENCING THE ACTIONS OF CORPUS LUTEUM EXTRACTS ON THE RABBIT'S UTERUS.

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In a previous communication [Robson and Illingworth, 1931] the effects of injection of various extracts of bovine corpora lutea into ovariectomized rabbits were described. The changes resulting in the uterus were studied in detail with special reference to the specific proliferation of the endometrium and inhibition of the reaction of the musculature to pituitrin. Evidence was presented which could best be explained on the assumption that the two reactions were due to different luteal hormones and that partial separation of these had been effected. It seemed at the time that crude extracts of the corpus luteum would always give both effects on the uterus, and Knaus [1930] had actually suggested using the inhibitory reaction as a means of standardizing extracts. Tausk *et al.* [1931] subsequently also showed that corpus luteum extracts did not cause both proliferation and inhibition with any degree of regularity, and that multiple doses of an extract might produce less inhibition than the original dose. They came to the conclusion that the correlation between the proliferation and inhibitory phenomenon was too small to justify the assumption that they were caused by the same factor. Clauberg [1931] suggested that the α hormone (œstrin) could prevent the development of the inhibitory reaction, and this point of view is further developed by his colleagues Hartmann and Störring [1931], although no exact experimental data are given.

During the last eighteen months we have obtained additional data. The effect of dosage on the development of the uterine reactions has been studied so as to allow of a more exact evaluation of the "separation" experiments. Methods have been evolved for the further purification of the corpus luteum extracts. Additional experiments also on the relationship of α (œstrin) to the changes brought about in the uterus by the luteal extracts have been performed.

TECHNIQUE.

Seventy-five mature female non-pregnant rabbits, each weighing about 2 kg., were used in these experiments. The animals were of various breeds, as we found it difficult to procure animals of the same breed in sufficient numbers; but we have satisfied ourselves that the results obtained were not due to variations occurring between different breeds of rabbits. The animals were oöphorectomized and the absence of pregnancy or pseudo-pregnancy was established at the operation. Injections were administered over a number of days. The animals were then killed by a blow on the occiput or by injection of air into an ear vein. One horn of the uterus was placed in Bouin's solution and sections prepared from it; the other was used for experiments *in vitro*. Ringer-Locke solution was used in all cases in 100 c.c. containers. Temperature (37·5° C.) and oxygen supply were maintained constant. Records on smoked drums were taken with lightly balanced lever. The dose of pituitrin used in the majority of experiments was 5 units. The contractility of the uterus was in all cases tested by the subsequent addition to the bath of 0·1 c.c. of 1/1000 adrenaline solution.

The standard extracts were prepared as follows: cow's ovaries were dissected within a few hours of the animals being slaughtered. Only solid corpora were used, and these were finely minced and extracted in the cold with two volumes of 95 p.c. ethyl alcohol for at least 24 hours in each extraction. The alcoholic extract was evaporated to dryness *in vacuo* and the residue extracted thoroughly with 100 c.c. portions of ether. The tissue, after alcohol extraction, was dried and extracted in a Soxhlet with ether, and the two ether extracts combined and evaporated to dryness. The viscous oil was then extracted with ethyl acetate. This on evaporation yielded a dark brown oil which solidified on standing. The yield was about 25 g. per kg. of corpora lutea.

RESULTS.

The results obtained with the standard extracts are grouped according to dosage in Table I. The degree of proliferation is shown in column 3; +++ represents a degree of proliferation equivalent to that observed on the 8th day of pseudo-pregnancy in an intact rabbit, while + indicates the minimal degree of proliferation clearly demonstrable. Column 4 shows the reaction of the musculature to pituitrin; + indicates that inhibition had developed, in other words, that pituitrin (5 units in 100 c.c. Ringer-Locke solution) was without effect or caused relaxation.

TABLE I.

Extract No. β	Animal No. Ra	Proliferation	Inhibition
Dose 1–2 c.c.			
15	59	+++	+
20	118	+++	–
23	158	+++	–
24	163	+++	+
35	245	++	–
36	253	++	+
41	264	+++	–
42	266	++	–
48	294	+++	–
54	310	++	+
Dose 2–3 c.c.			
22	122	++	+
29	224	++	–
30	208	+++	+
35	242	+++	–
50	299	+++	+
51	308	+++	+
52	303	+++	–
53	302	++	+
58	321	+++	+
Dose > 3 c.c.			
29	230	+++	–
41	265	++	+
44	276	+++	+
44	298	+++	+
24	163	++	+
48	300	+++	+
35	246	+++	+
52	304	+++	–

It will be seen that in all cases the extract caused some degree of proliferation. Inhibition, however, was not obtained in all the experiments, but it will be noticed that the incidence of inhibition increases with the dose administered. This is more clearly shown in Table II which correlates the dosage with the percentage of experiments in which both effects were obtained. It will be seen that, while a dose of 1–2 c.c. caused inhibition in only 40 p.c. of experiments, the larger dosage (*i.e.* doses greater than 3 c.c.) were successful in causing inhibition in 75 p.c. of experiments.

TABLE II.

Dosage c.c.	No. of animals	Proliferation and inhibition p.c.	Proliferation only p.c.
1–2	10	40	60
2–3	9	66·6	33·4
3	8	75	25

As it had been found previously that purification of the extracts by partitioning between volatile media (to which we shall make further

reference in the discussion) was attended by some degree of dissociation of the two effects, further methods of purification were evolved in the hope that a more definite separation would result.

(i) *Partition between* 66 *p.c. acetic acid and petroleum ether* (B.P. 40–60° C.). In a preliminary experiment 2 c.c. of extract β18 were dissolved in 10 c.c. petroleum ether and the solution extracted thoroughly with like portions of 66 p.c. acetic acid. The solvents separated fairly readily and the extraction was continued until the acetic acid remained colourless after shaking with the petroleum ether solution.

On evaporating the acetic acid fraction to dryness under reduced pressure, a greenish brown gum remained which, when injected into rabbit 93, caused good proliferation (+++) and complete inhibition. The petroleum ether fraction had retained none of the active substance.

While no separation of the active factors was effected, the volume was reduced very considerably and a colloidal solution in water produced. Therefore, the method was considered to be of value in so far as purification of the crude extracts was concerned and has been used in a number of experiments. In no case has the preparation produced been desiccated, so that dry weights for the unit cannot be given, but 1 g. of the "gum" contains about 20 rabbit units.

TABLE III. To show the effect of the acetic acid fraction on the uterine endometrium and muscle.

β	Animal No. Ra	Proliferation	Inhibition
18	93	+++	+
18	96	+++	–
20	107	++	–
36	254	++	–
48	300	+++	+
54	310	+++	+
55	309	+	+

(ii) *Partition between methyl alcohol and petroleum ether* (B.P. 40–60° C.). 2 c.c. of extract β23 were dissolved in petroleum ether and the solution extracted twelve times with methyl alcohol, until, that is, the alcoholic phase remained colourless. The combined alcohol fractions were washed once with a small volume of petroleum ether and evaporated to dryness *in vacuo*. The residue was dissolved in a convenient quantity of maize oil and injected into Ra 149. Section of the uterus showed good proliferation, while a marked contraction followed the addition of pituitrin to the uterus *in vitro*. This experiment was repeated with β24 and β25. The results are shown in Table IV.

TABLE IV. To show the effect of the methyl alcohol fraction on the uterine endometrium and muscle.

β	Animal No. Ra	Proliferation	Inhibition
23	149	+++	–
23	159	+++	–
24	164	++	–
25	182	++	–

(iii) *Partition between* 45 *p.c. ethyl alcohol and petroleum ether* (B.P. 40–60° C.). Separation of the solvents was extremely slow and tedious, and the addition of a drop of HCl was without effect. The alcohol fraction on evaporation yielded a minute amount of inactive material, while the petroleum ether fraction had completely retained its activity as shown by injection into Ra 60.

(iv) *Partition between* 50 *p.c. ethyl alcohol and chloroform.* In this experiment, which was carried out as described above, the chloroform fraction on injection into Ra 74 produced good proliferation and complete inhibition, while the portion soluble in alcohol was without activity.

(v) *Partition between* 50 *p.c. ethyl alcohol and benzene.* Separation of the solvents was exceedingly slow owing to the formation of an emulsion. The product obtained from the alcohol fraction (used in aqueous suspension) was almost inactive, whereas the benzene fraction appeared to have lost very little of its activity.

(vi) *Partition between* 66 *p.c. acetone and petroleum ether.* 2 c.c. of extract β16 were dissolved in petroleum ether and the solution extracted with an equal volume of 50 p.c. acetone. An emulsion was formed and therefore the concentration of the acetone was increased to 66 p.c. when separation of the solvents took place readily. On injection of the acetone-soluble fraction into Ra 79 it was found to be without activity, while the petroleum ether fraction caused good proliferation and inhibition when injected into Ra 80.

Modification of methods of initial extraction. During the summer months of 1931 it was found difficult to obtain supplies of corpora lutea from Glasgow in sufficient amount and consequently the glands were obtained from Holland preserved in acetone. On arrival in the laboratory the acetone was drained off the corpora, the tissue minced and extracted with 95 p.c. alcohol twice for 24 hours. The solvents were combined, evaporated *in vacuo* and the preparation carried out as hitherto described.

All the extracts prepared from these supplies were found to be completely inactive, and on enquiry being made in Holland it was found that the corpora lutea had been stored in acetone for some considerable time.

In view of this, therefore, the effect of acetone extraction was investigated more carefully as follows: Fresh corpora lutea were finely minced and extracted in the cold for several days and in some cases for several weeks. The acetone was filtered off and evaporated *in vacuo*. The residue was extracted with ether and the ether soluble material used for injection. The results obtained with a number of such extracts are shown in Table V.

TABLE V. To show the effect of an acetone extract of bovine corpora lutea on the uterine endometrium and muscle.

Period of extraction days	β	Weight of corpora lutea g.	Animal No.	Proliferation	Inhibition
4	31	500	Rb 3	+++	–
4	33	300	Rb 5	+++	–
6	37	250	Ra 257	++	–
2	39	240	Ra 260	++	+
2	43	275	Ra 273	+	+
40	46	150	Ra 305	+++	–

It will be seen that although large quantities of tissue were extracted (the minimal amount being equivalent to 4 c.c. standard extract) and that good proliferation resulted in five out of six experiments, yet inhibition was only obtained in two out of six.

Extraction with methyl alcohol was investigated, but little evidence was obtained to show that it had any advantage over ethyl alcohol as an extracting medium. It seemed rather, that prolonged treatment with this solvent inactivated the inhibitory factor. Extraction of corpora lutea for 2–3 days produced an extract which caused both proliferation and inhibition when injected in the usual way, while the extract obtained after treatment of the tissue for periods of 10 days or more caused only proliferation.

In view of the statement of Fevold, Hisaw and Leonard [1932] to the effect that by means of acid alcohol extraction and subsequent fractionation, it is possible to produce a number of active principles of the corpus luteum, it was decided to apply their method in an attempt to produce extracts which would bring about one or the other of the effects described herein.

Fresh bovine corpora lutea were finely minced and refluxed with two volumes of acid alcohol (98 c.c. ethyl alcohol + 2 c.c. HCl) twice, for 1 hour in each extraction. The tissue was completely disintegrated by the boiling acid alcohol which was removed by filtration through muslin; the dark brown solution was neutralized with sodium bicarbonate and the precipitate which formed was redissolved and the neutralization repeated.

The solution was evaporated to dryness *in vacuo* at a temperature below 45° C. and the residue extracted with alcohol; this was then removed under reduced pressure and the residue extracted with ether. Phosphatides were precipitated with acetone and the fraction soluble in acetone injected into Ra 313 and Ra 315. In each case the uterus showed slight proliferation (+) on section and no inhibition. A preparation from the same batch of corpora lutea carried out in the normal manner produced an extract which caused good proliferation with complete inhibition. This was repeated with a fresh batch of corpora lutea, and the extract obtained by the method of Hisaw was found to be almost inactive (Ra 329; proliferation +, inhibition negative). In every case the amount of extract administered to each animal was slightly greater than the usual dose of standard extract.

Relationship of α (œstrin) to the action of the luteal extract. It has been previously shown that the administration of α hormone (3000 m.u.) concomitantly with corpus luteum extract in oöphorectomized animals does not interfere with the development of the typical endometrial picture (progestational proliferation), nor with the inhibitory reaction.

It still was possible that the administration of α over a more prolonged period before the injections of corpus luteum extract would influence the subsequent effect of the extract on the uterine musculature as has been actually suggested by Hartmann and Störring [1931]. A series of experiments was carried out to investigate this possibility.

A number of animals were injected with 10 m.u. of an oily preparation of α per day, the first injection being given on the day following oöphorectomy. The α hormone was administered for 6 or 7 days and this was followed by the injection of corpus luteum extract, in doses similar to those used in previous experiments, over the usual period of 3 days. The animals were then killed and the state of the uterine endometrium and muscle determined. The results are figured in Table VI.

TABLE VI. To show the effect of α (œstrin) on the reaction of the uterine muscle and endometrium to the corpus luteum extract.

β	Dose c.c.	Proliferation	Inhibition	Animal No. Ra
35	2·7	+ + +	+	243
36	2·4	+ + +	+	262
36	2·4	+ + +	+	275
42–44	1·8	+ + +	−	287
42–44	3·6	+ + +	−	290
42–44	3·6	+ + +	−	291
48	3·6	+ + +	+	319
50	3·6	+ + +	+	322
52	3·6	+ + +	−	323

It will be seen that in each of the nine animals so treated a positive endometrial reaction was obtained. In addition, five out of the nine (or 55 p.c.) showed inhibition of the reaction to pituitrin. This result is not significantly different from that obtained in experiments where no α was given and where the injection of corpus luteum extract commenced immediately after oöphorectomy. It must therefore be concluded that under the given experimental conditions, the α hormone does not adversely affect the development of the inhibitory reaction.

Discussion.

The experiments described in this paper show in the first instance, that when an extract of the corpus luteum is injected into oöphorectomized rabbits some degree of correlation exists between the effects of such an extract on the uterine endometrium and muscle respectively; the degree of correlation is dependent on the dosage and increases in proportion to the amount administered. Nevertheless even with large doses there is still a significant number of experiments in which the uterus showed a positive reaction to pituitrin.

Tausk *et al.* [1931] have recently obtained similar results working on immature animals injected for 7–8 days with α (œstrin) previous to the administration of the luteal extracts and record the actual sensitivity of the uterine muscle to pituitrin in quantitative terms. No data are given for the sensitivity of animals treated with œstrin alone, so that apparently they have no standards of comparison. Our experience (with oöphorectomized and non-oöphorectomized mature animals) suggests that there exist great variations in the reactivity of the uterine muscle to pituitrin in the absence of any luteal activity, and for this reason we have attached significance only to those results in which the uterus failed to react to large doses of pituitrin. We are not satisfied that the failure of the uterus to react to small doses of pituitrin is necessarily due to an action of the luteal hormones.

From the given data it seems possible that an additional factor is involved in the production of the inhibitory reaction. Clauberg [1931] has indeed suggested that the amount of α administered is the decisive factor in determining whether inhibition develops, and both the α present in the extracts and the amount administered in the preliminary period in immature animals have to be considered; this point of view is further developed by his collaborators Hartmann and Störring, although no exact experimental data are given. This hypothesis is not supported by

our experimental material, for it has been shown that the administration of doses of α similar to those used by Clauberg *et al.*, and over a similar period, produces no appreciable effect on the development of inhibition. It must further be emphasized that our own extracts contain little α hormone ($<$ 1 m.u. per rabbit unit corpus luteum extract), so that variations due to the α content of the preparations have, to all practical purposes, been eliminated. This conclusion is supported by experiments published elsewhere [Robson, 1932], where it is shown that the injection of 10 m.u. of α per day not only does not interfere with the development of the inhibitory phenomenon, but is actually necessary for the action of the luteal hormone on the uterine musculature in non-oöphorectomized animals injected with large doses of anterior lobe tissue.

The evidence we have previously advanced in which the corpus luteum and α hormones were administered simultaneously in oöphorectomized animals, also shows that α does not interfere with the inhibitory reaction.

Although the two reactions of the uterus (proliferation and inhibition) occur together in the majority of experiments, there is nevertheless evidence that they may be due to separate factors. In a previous paper [Robson and Illingworth, 1931] the results of experiments in which extracts of the corpus luteum were partitioned between 50 p.c. alcohol and petroleum ether were given, and the data now presented make it possible to estimate more accurately their significance. The results obtained are shown in Table VII.

TABLE VII.

		50 p.c. alcohol			Petroleum ether		
β	Dose c.c.	Animal No.	Proliferation	Inhibition	Animal No.	Proliferation	Inhibition
12	3	36	+	++	39	++	−
12	3	Not used			46	+++	−
12	3	42	++	−	41	+++	+
16	2	65	++	+	63	+++	−
16	2	67	+++	+	66	+++	+
16	2	70	++	+	69	+++	−
18	2·5	90	++	−	Not used		
14	3	49	++	+	48	+	−
14	3	50	+++	+	54	+++	−
14	2	56	+++	−	82	+++	+
17	1·5	81	+	−	84	++	−
17	1·5	85	++	+			

It will be seen that with the fraction soluble in 50 p.c. alcohol inhibition was obtained in seven out of eleven experiments, *i.e.* 63 p.c. of cases: with the petroleum ether fraction inhibition was only obtained in three out of ten experiments, *i.e.* 30 p.c. of cases. According to expectation the dose used should have given inhibition in 50 p.c. of experiments, the

results therefore suggest that the 50 p.c. alcohol phase contains about twice as much of the inhibitory factor as the petroleum ether fraction, yet the degree of proliferation with the petroleum ether fraction is more marked than that given with the alcoholic fractions. These results suggest to us that the two actions on the uterus can be referred to separate factors of the corpus luteum, but definite proof will only be obtained when a complete separation is accomplished.

While no advance has been made in the actual separation of the active factors of the corpus luteum (should two exist) a method has been evolved whereby a considerable purification of the standard extract can be quickly and conveniently effected.

By distributing the extract between 66 p.c. acetic acid and 40–60° C. petroleum ether, the weight of the extract is reduced from about 2 g. to 50 mg., and by more thorough extraction may be further reduced to 30 mg. without loss of activity. Allen's method [1930], although effective in obtaining a similar degree of purity, is long and tedious and considerable losses are sustained.

The effect of methyl alcohol extraction and separation is not clearly understood. In less than 20 p.c. of the total number of cases in which methyl alcohol was used, were both proliferation and inhibition obtained. A similar effect was obtained with acetone. The weights of tissue originally extracted with these solvents were, as a rule, considerably greater than those which yielded 1 rabbit unit by the standard method. The effect on the endometrium showed that active extracts were being produced. These experiments therefore appear to support the point of view that two factors are present in the corpus luteum, although, again, no complete separation was effected in these experiments.

The method of extraction described by Fevold, Hisaw and Leonard [1932] was investigated and found to produce an extract devoid of almost all activity. In view of the fact that these authors claim to obtain 30 rabbit units of the proliferative hormone per kg. of sow corpora lutea, and we adhered to their method exactly, our results can only be explained by the fact that bovine corpora lutea were used by us.

Standardization. Whatever decision will ultimately be arrived at as regards the existence of two luteal factors, it seems to us fairly evident that the inhibitory reaction is not a satisfactory test for the progestational hormone of the corpus luteum.

The progestational reaction of the endometrium gives more reliable and constant information as to the hormone content of a given extract. We have arrived at this conclusion from experiments performed on

mature oöphorectomized animals using a modification of the method originally devised by Corner and Allen [1929]. We have had no experience of the test as performed on immature animals, but from the published data cannot see that it has any advantages over that performed on the mature rabbit.

Summary.

The action of standard extracts of the corpus luteum on the uterus of mature oöphorectomized rabbits was determined, with special reference to the effects on the endometrium (progestational proliferation) and the muscle (inhibition of the pituitrin reaction).

With a dose of 1 c.c. or more of the standard extract, progestational proliferation was obtained in nearly all cases; the frequency with which inhibition occurred varied with the dose injected, a larger dose being more often followed by the inhibitory reaction; even with comparatively large doses, however, 25 p.c. of the experiments showed the endometrial reaction without the effect on the muscle.

A method is described which allows of the further purification of the standard extract; the material so obtained produces the full effect on the endometrium in doses of less than 50 mg. and gives a colloidal solution when shaken up with water.

The effect of the α hormone (œstrin) on the reactions of the uterus to the corpus luteum extract has been further investigated. The α hormone does not appear to interfere with the development of the inhibitory reaction.

The evidence suggesting that the inhibitory reaction is due to a separate luteal hormone is discussed.

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THE ACTION OF PITUITARY POSTERIOR LOBE EXTRACTS ON DIFFERENT PARTS OF THE CIRCULATORY SYSTEM.

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THE action of pituitary posterior lobe extracts on the circulation has received considerable attention, but has not been fully explained. All investigators are agreed that an intravenous injection of pituitrin produces constriction of the splanchnic vessels with a rise of systemic blood-pressure, but opinions differ as to how the other parts of the vascular system and the heart are influenced. Difficulties may exist in the fact that different species of animals do not behave in the same manner, so that it is not possible to transfer the results obtained from one to the other; on the other hand, they may arise from the fact that different investigations have been carried out under experimental conditions not altogether comparable with one another. Results obtained from observations on isolated organs, from perfusion of isolated parts of the vascular system or from the heart-lung preparation, cannot without reserve be applied to the intact animal. When therefore, for example, Dale [1909] or Gunn [1926] finds with the isolated heart that pituitrin constricts the coronary vessels, or when Anrep and Stacey [1927–8], Roessler [1930] and others describe its action on the heart-lung preparation, these observations cannot without further evidence be transferred directly to the conditions which obtain in the intact animal. It is further necessary, in the case of any effect on isolated tissues or organs, to be certain that it is due to an essential hormone in the extract and not to accidental accessory constituents, such as histamine. Further, now that it is known that the extract of the pituitary posterior lobe contains more than one active principle, it is important to know, especially with respect to certain effects, which of the known principles is responsible.

The present paper is an attempt to explain the effect of pituitary preparations upon the circulation by describing in the first place investigations on anæsthetized but otherwise intact animals, and in the second place experiments on isolated tissues or perfused organs, in order to analyse the results obtained on the whole animal. The effects seen in different species of animals have been compared in order to gain a wider basis on which to criticize the results.

The commercial extract known as pituitrin and the separated pressor and oxytocic principles, pitressin and pitocin, have all been used in different parts of the investigation.

THE ACTION OF PITUITARY EXTRACTS ON THE HEART AND PULMONARY CIRCULATION OF THE RABBIT, THE CAT AND THE DOG.

Rabbit.

Method. The experiments were made on animals anæsthetized with urethane (1·5 g. per kg.). The systemic arterial pressure was measured in the carotid with a mercury manometer. In order to measure the pressure in the auricles and in the pulmonary artery the chest was opened by the removal of the upper three or four ribs from their sternal ends. The pressure in the left auricle was measured by inserting a cannula either into the left auricle directly or through a pulmonary vein; in the right auricle by a long cannula inserted through the jugular vein, and in the pulmonary artery by Schafer's method. The pressure was recorded in millimetres of half-saturated sodium sulphate solution.

Results. The most striking effect of an intravenous injection of pituitrin in the rabbit is produced by its action on the heart. This is well shown by the volume curves obtained with the air oncometer. A small dose of 0·1 unit causes only a small diastolic dilatation by which the output of the heart is influenced probably only to a negligible degree. A somewhat larger dose of 0·3 unit leads to a more definite dilatation, the cardiac output per beat being definitely diminished for about half a minute, whilst the heart rate undergoes little change. Larger doses such as 0·5 unit profoundly weaken systole; the heart dilates and the rate of beat is diminished (Fig. 1). This is in conformity with many previous investigations [Tigerstedt and Airila, 1913; Börner, 1916; Müller, 1917; Wolfer, 1922].

Small doses of pituitrin—less than 0·3 unit—administered to the isolated rabbit's heart perfused with Ringer's solution by Langendorff's method, may diminish the coronary flow to one-half without influencing the heart beat, as recorded in the usual manner. These observations are in conformity with those of Anrep, who in the heart-lung preparation of the dog found that there is no relation between the

rate of the coronary flow and the action of the heart [Anrep and Bulatao, 1925; Anrep and Stacey, 1927–8]. It may be mentioned, however, that Bodo [1927] in experiments on the heart-lung preparation found that constriction of the coronary vessels was always accompanied by simultaneous dilatation of the ventricles; and Roessler [1930] thinks that the injurious effect of pitressin on the dog's heart is mainly due to constriction of the coronary vessels. After larger doses of pituitrin (0·5 unit and more) the constriction of the coronary vessels in the isolated

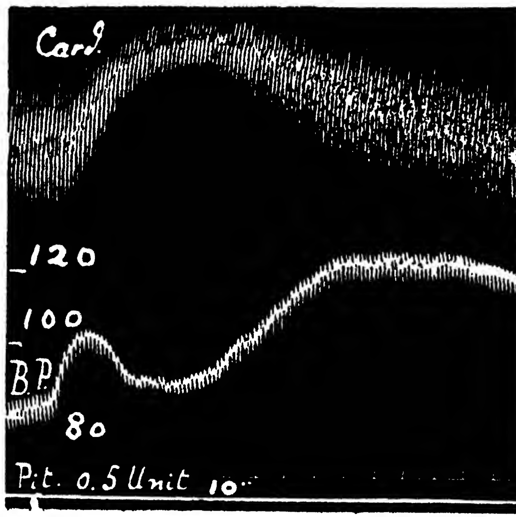

Fig. 1. Rabbit. Urethane. Cardiometer. Dilatation of the heart after 0·5 unit pituitrin. Time = 10 sec.

rabbit's heart occurs simultaneously with a decided decrease in frequency and a diminution in the size of the contractions. Thus the result of these experiments on the isolated rabbit's heart speaks in favour of a direct action of pituitrin on the heart muscle itself, besides its constrictor effect on the coronary vessels.

The reaction of the heart to pituitrin explains the peculiar effect on the carotid blood-pressure. Tracings of this show an initial rise followed by a distinct fall, which is then followed by a second and larger rise. The fall occurs simultaneously with the diastolic expansion and is clearly due to it (Fig. 1). The injured heart cannot overcome the increased systemic arteriolar resistance, and, as a result, the arterial pressure falls

to a certain extent. The heart, however, quickly recovers and the blood-pressure rises again.

The pressures in the left and in the right auricle fall when a small dose of pituitrin is injected. In one experiment a dose of 0·2 unit caused a fall of pressure of 10 mm. of half-saturated sodium sulphate solution in the right auricle and a fall of 12 mm. in the left auricle. These effects are probably due to the strong constriction of the coronary vessels, leading to a diminution of blood flow to the right auricle, so that the

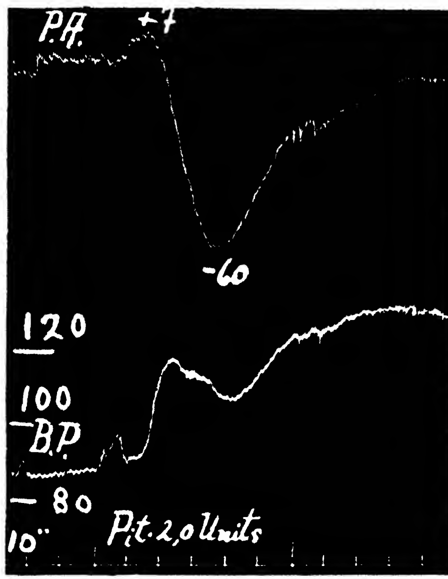

Fig. 2. Rabbit. Urethane. Fall of pulmonary artery pressure (P.A.) after 2·0 units pituitrin. Time = 10 sec.

right ventricle sends a smaller amount of blood through the lungs into the left auricle. There is no back pressure from the ventricles, since the contractile power of the heart is not materially affected by these small doses of pituitrin.

If larger doses are injected, the heart beat is weakened and the back pressure of the dilated ventricles increases the pressures in the auricles.

The pressure in the pulmonary artery always falls after injections of pituitrin, no matter what the dose may be (Fig. 2), as also stated by Sharpey-Schafer and Macdonald [1926–7]. When the dose is small, this effect is probably due to the decrease in the flow through the coronary

vessels: the right heart receives less blood. After larger doses an increase of the pressure of the pulmonary artery might be expected, because the raised pressure in the left auricle augments the resistance in the pulmonary circulation. But these larger doses cause a profound weakening of the cardiac muscle. Thus the right ventricle does not only receive less blood by the constriction of the coronary vessels, but even sends out less blood than it receives. That again explains further the fact, which might appear paradoxical, that simultaneously with the fall of pressure in the pulmonary artery the pressure in the right auricle increases slightly.

The action of pituitrin on the pulmonary circulation of the rabbit depends therefore upon two main factors: (1) the depression of cardiac muscle, (2) the constriction of the coronary vessels.

Cat.

Method. The animals were anæsthetized with A.C.E. and urethane. In order to measure the pressure in the pulmonary artery, a cannula was tied into a branch on the hilus of the right upper or middle lobe. The lung volume was recorded by the Brodie-Dixon method with an air tambour connected to an oncometer enclosing the middle lobe of the right lung. The bronchus of this lobe was ligatured.

Results. From the experiments dealt with in the first part of this paper it is evident that in rabbits the action of pituitrin on the heart is of great importance for its effect on the pulmonary circulation. It therefore seems best, in describing the action of pituitrin on the cat's circulation, to begin by discussing its effects on the heart.

The investigations of previous workers [McCord, 1911; Biedl, 1913; Börner, 1916], most of which were published some time ago, show that the cat's heart is much more resistant to pituitrin than the rabbit's heart. I can confirm this. Fig. 3 shows the effect of 1·0 unit vasopressin on the isolated cat's heart. It can be seen clearly from it that the coronary flow is increased and the contractions of the heart enlarged: the heart is therefore stimulated. The same dose administered to the heart of a rabbit would lead to a considerable decrease of the coronary output and a reduction in the amplitude and frequency of the heart beat. Larger doses—3·0 units—either do not change the action of the cat's heart or cause a slight diminution in the contractions. The coronary vessels remain uninfluenced. The experiments with the cardiometer confirm these results. 1·0 unit of pituitrin or pitressin is almost without any effect, large doses produce only a small diastolic expansion. It can therefore be concluded, that small doses of pituitrin or pitressin—not

exceeding 1·0 unit—which would definitely damage the rabbit's heart, either leave the cat's heart entirely untouched or even lead to an improvement of its action, and that very large doses—up to 3·0 or 4·0 units—may slightly weaken the cat's heart, although the effect is still small compared with that seen in the rabbit's heart. The auricular pressures either remain unchanged or fall after small doses of pituitrin such as 0·5 or 1·0 unit. The fall of auricular pressure in the rabbit has already been attributed to a diminution in the blood flow to the right auricle, caused by constriction of the coronary vessels. In the isolated heart of the cat pituitrin has no such effect on the coronary vessels, and it is therefore probable that direct stimulation of the ventricles is the

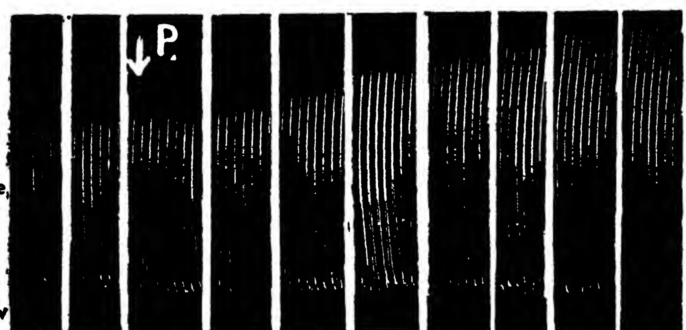

Fig. 3. Cat. Isolated heart (Langendorff's method) 1·0 unit pitressin (↓ *P.*) augments the coronary flow and stimulates the action of the heart. Each sector corresponds to 30 sec.

main cause of the small fall of auricular pressures which usually occurs. Large doses of pituitrin cause a rise of auricular pressure in the cat. No direct comparison has been made of the relative magnitude of the effect on the two auricles, but in a number of experiments in which the pressure in the right auricle was recorded, the effect of pituitrin was always larger than that seen in other experiments when the pressure in the left auricle was recorded. The rise in auricular pressure may be accounted for, as in the case of the rabbit, partly by weakening of the ventricles, but it will be seen later that there are two other factors which probably contribute to the rise of auricular pressure after large doses in the cat but not in the rabbit. In contrast to the effects seen in the rabbit the pressure in the pulmonary artery of the cat is invariably increased by the injection of pituitrin (Fig. 4). This effect might have

been due to vaso-constriction of the pulmonary vessels, but no such action could be demonstrated in the isolated and perfused lungs of the cat, although these vessels showed definite reactions to injections of small doses of adrenaline and histamine.

The fact that simultaneously with the rise of the pressure in the pulmonary artery the volume of the lung is augmented (Fig. 5), points to the suggestion that pitressin causes an increased blood supply to the lungs. This increase of blood flow to the cat's lungs may be due to two

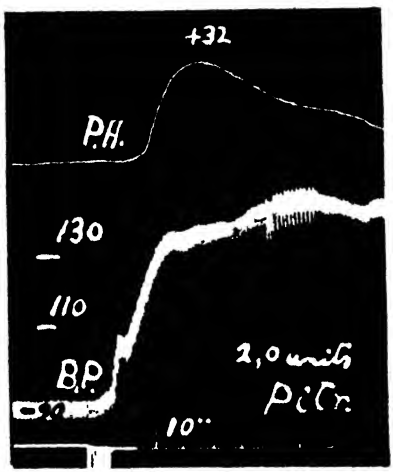

Fig. 4.

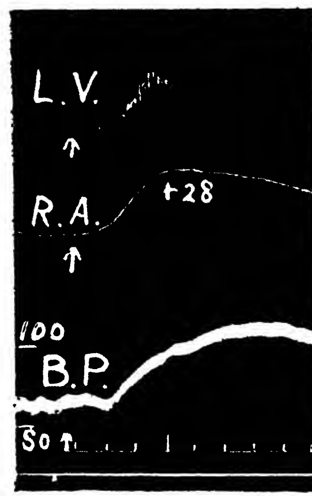

Fig. 5.

Fig. 4. Cat. Urethane. Rise of pulmonary artery pressure (P.A.) after 2·0 units pitressin. Time = 10 sec.

Fig. 5. Cat. Urethane. Increase of lung volume (L.V.) and rise of pressure in the right auricle (R.A.) after 2·0 units pituitrin. Time = 10 sec.

causes: (1) Anrep and Bulatao [1925] found in investigations concerning the distribution of blood flow between the coronary and systemic vessels, that an increase in the systemic resistance produces an increased blood flow through the coronary vessels. Since the coronary vessels of the isolated cat's heart are not constricted by pitressin and may even be dilated, it is probable that in the whole animal the constriction of the systemic vessels leads to an actually increased coronary flow. As a result of this, more blood than normal will flow to the right heart and to the lungs; (2) Berry and Daly [1932], in perfusing the lungs simultaneously through the pulmonary artery and the posterior bronchial

artery, which latter takes its origin from the aorta, have shown that a rise of pressure in the bronchial artery causes a corresponding rise of pressure in the pulmonary artery and an increased output from the lung veins into the left auricle. Thus the augmented peripheral resistance, as caused by the injection of pitressin, might possibly increase the blood supply to the lungs through the bronchial arteries.

The action of pituitrin on the pulmonary circulation of the cat therefore depends upon the increase of blood supply to the lungs caused (1) by an active dilatation of the coronary vessels—after small doses, (2) by a passive increase in coronary flow which is due to the increase in systemic resistance—after large doses, and perhaps (3) by an increased blood flow through the bronchial circulatory system into the lungs, which also is caused by the augmented peripheral resistance.

Dog.

Method. The experiments on dogs were performed under chloralose anæsthesia (0·1 g. per kg. intravenously). The pressure in the pulmonary artery was measured in a side branch on the hilus of the right upper or middle lobe; the pressures in the left and right auricle by tying cannulæ direct into the appendices. The lung volume was recorded as described for the cat.

Results. If pituitrin or pitressin is injected intravenously into a dog, the rise in arterial blood-pressure is never so pronounced and immediate as in the rabbit and in the cat. Sometimes even a fall after an initial rise may be obtained. The carotid curve shows slowing of the pulse and irregularities in the action of the heart. In agreement with the results obtained by previous workers [Mautner and Pick, 1929; Kolls and Geiling, 1924; Roessler, 1930] it was found that pituitrin dilates the ventricles and constricts the coronary vessels: like the rabbit's heart, and in contrast to the heart of the cat, the dog's heart is very much injured by pituitrin.

The pressure in both auricles is raised, but the difference in the magnitude of the rise of pressure in the right and the left auricle is just the reverse of that found in the cat: while the pressure in the right auricle shows only a small rise, that in the left auricle is very considerably increased, sometimes tenfold or more (Fig. 6). Thus it appears that the left ventricle of the dog is injured by pitressin to a higher degree than the right ventricle, as also was concluded by Roessler from his experiments with the heart-lung preparation. The outflow from the lung veins into the left auricle is thus retarded, and one would expect that the pressure in the pulmonary artery would rise against the in-

creased resistance. But in spite of the increased pressure in the left auricle the pressure in the pulmonary artery always shows a pronounced fall (Figs. 7 and 8). It is obvious in so far as the heart is concerned that there are two factors tending to change the pulmonary arterial pressure in an opposite direction: (1) the back pressure from the left auricle tends to raise it, (2) the constriction of the coronary vessels, followed by a diminished blood flow to the right auricle, tends to lower it.

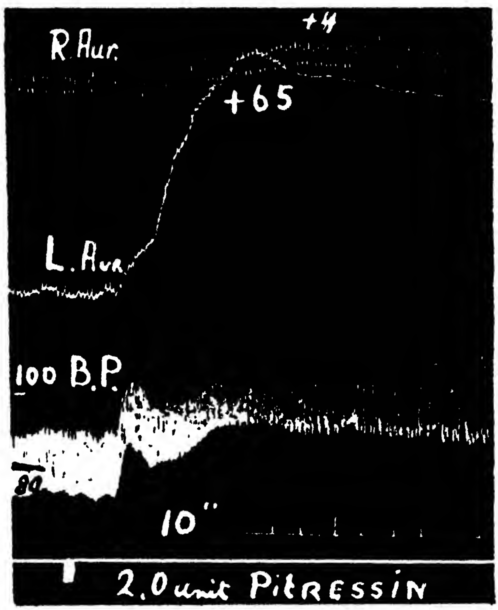

Fig. 6. Dog. Chloralose. Rise of pressure in the right and left auricle after 2·0 units pitressin. Time = 10 sec.

That after the injection of pitressin the blood supply to the lungs is actually diminished is proved by the decrease in lung volume (Fig. 9). The constriction of the coronary vessels, however, cannot be the only cause for the diminished blood flow to the lungs. From experiments on the heart-lung preparation I can confirm Roessler in that a second dose of pitressin has almost no effect on the coronary vessels. In one of my experiments for example the first injection of 3·0 units of pitressin diminished the flow through the coronary vessels from 58 c.c. per min. to 20 c.c. per min.; the same dose, injected 22 min. later, had practically no effect on the coronary vessels, producing only a diminution of flow

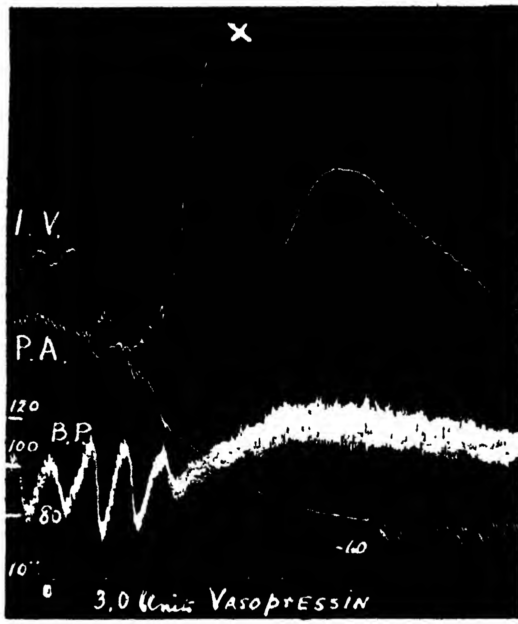

Fig. 7. Dog. Chloralose. Effect of 2·0 units of pitressin on the pressure in the pulmonary artery (P.A.) and the intestinal volume (I.V.) X = bellows lowered.

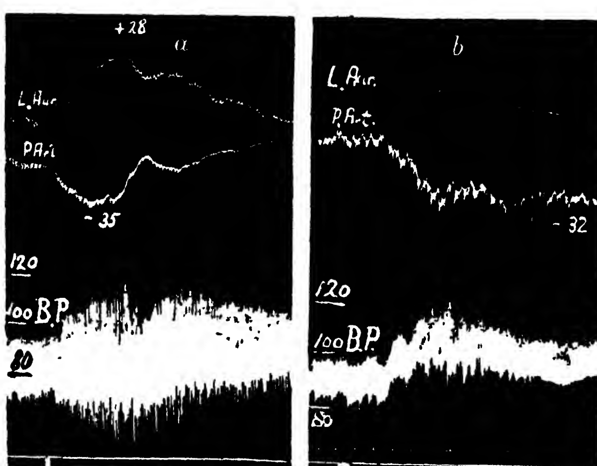

Fig. 8. Dog. Chloralose. (*a*) Injection of 1·0 unit pitressin: fall of pulmonary artery pressure (P.Art.); rise of left auricular pressure (L.Aur.). (*b*) 22 min. later; injection of 1·0 unit pitressin: fall of pulmonary artery pressure (P.Art.); slight fall of left auricular pressure (L.Aur.).

from 54 c.c. per min. to 50 c.c. per min. The coronary vessels therefore are refractory against a second injection of pitressin, the dilatation of the heart does not take place after the second dose and the pressure in the left auricle remains unchanged or even shows a slight fall (Fig. 8 *b*).

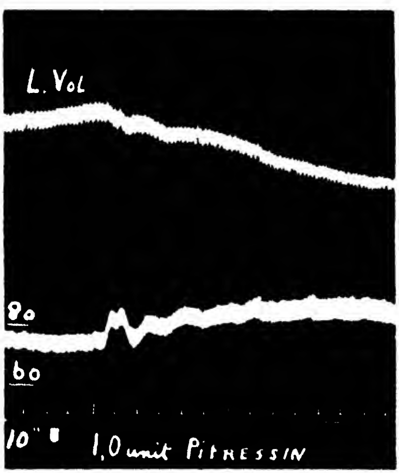

Fig. 9.

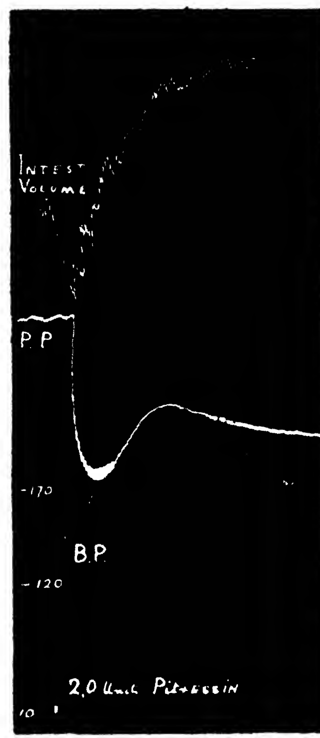

Fig. 10.

Fig. 9. Dog. Chloralose. Diminution of lung volume (L.Vol.) after 1·0 unit of pitressin. Time = 10 sec.

Fig. 10. Dog. Chloralose. Effect of 2·0 units of pitressin. Increase of intestinal volume and fall of portal pressure (P.P.). The rise of the systemic arterial pressure (B.P.) is only partially recorded.

In spite of this, the pressure of the pulmonary artery falls also after the second injection (Fig. 8 *b*). From this we can conclude that other parts of the circulatory system play an important part in the action of pitressin on the pulmonary circulation, and I think one is justified in assuming that the cause for the fall of pulmonary arterial pressure after a second injection of pitressin is the diminution in the return of blood

from the arterial side to the venous side of the vascular system. This view is supported by the fact that the pressure in the femoral and jugular vein falls if pitressin is injected.

It is therefore not surprising that in the heart-lung preparation the pressure in the pulmonary artery invariably rises after pitressin has been injected, because here the whole peripheral vascular system is ligatured off and the diminution of blood flow to the right heart, as caused by the constriction of the coronary vessels, is probably largely masked by the constant inflow from the venous reservoir. Thus the increased pressure in the left auricle is the only factor in determining the height of pressure in the pulmonary artery, which must therefore rise with a first, and remain unchanged with a second injection of pitressin, since heart and coronary vessels are now desensitized and the left auricular pressure does not rise a second time.

The action of pituitrin on the pulmonary circulation of the dog therefore depends upon the diminished blood supply to the lungs as caused (1) after the first injection by constriction of the coronary and systemic vessels, (2) after the second injection by constriction of the systemic vessels only.

THE ACTION OF PITUITARY EXTRACTS ON THE MESENTERIC AND PORTAL CIRCULATION OF THE CAT AND THE DOG.

In confirmation of the results obtained by previous investigators [Miura, 1925; Clark, 1928], it was found that the portal pressure in cats and dogs, as measured in the splenic vein, shows a distinct fall if pituitrin or pitressin is injected into the general circulation. This corresponds with the observation of Mautner and Pick [1929], that the volume of the liver as measured in the living animal is diminished.

The fall of portal pressure and the diminution in liver volume is usually attributed to the fact that pituitrin constricts the splanchnic vessels and thus diminishes the blood supply to the portal system. This view is supported by the early observations of Oliver and Schafer [1895], that in the cat the intestinal volume is diminished after an injection of pituitary extract. Thus it seems that in the cat a diminished blood supply to the portal system, caused by constriction of the splanchnic vessels, might be sufficient to explain the fall in portal pressure and the diminution in liver volume. In the dog, however, this explanation does not apply, since in the dog (Fig. 10) simultaneously with the fall in portal pressure the intestinal volume after a short decrease is augmented.

The explanation of the fact, that in the dog the liver shrinks and the pressure in the portal vein falls, although the blood flow to the splanchnic area is increased, might be found either in that pituitrin constricts the mesenteric veins, thus leading to an accumulation of blood in the intestinal vessels and a diminution of blood flow into the portal vein and to the liver, or in that it has a dilator effect on the outflow side of the portal system, *i.e.* on the hepatic veins, thus diminishing the resistance, against which the blood from the portal system flows into the vena cava.

Both possibilities were examined by performing perfusion experiments (*a*) on isolated mesenteric vessels and (*b*) on the isolated liver.

(*a*) *Perfusion of isolated mesenteric vessels of cats and dogs.*

Method. The animals were anæsthetized with ether-chloroform and bled from the carotid. The blood was defibrinated and the perfusion apparatus filled. Cannulæ were then tied in the superior mesenteric artery and the splenic vein, and the whole small intestine separated and divided between two ligatures. The blood in the mesenteric vessels was washed out by perfusion through the splenic vein with hot Ringer. Then the intestine was resected at the insertion of the mesentery, so as to perfuse *either* the isolated mesenteric arteries *or* the veins in the reversed direction, the blood escaping from the cut arterioles and venules, as the case might be, without passing through capillaries [Dale and Richards, 1919]. The artery was perfused by means of the Dale-Schuster pump, the vein by hydrostatic pressure (in other experiments also the artery perfusion was performed by hydrostatic pressure). By means of clips it was possible to perfuse separately either the artery or the vein. The outflow was recorded by the method described by Gaddum [1929].

Results. The mesenteric arteries both of cats and dogs were shown in this way to be constricted by pitressin. This effect of pitressin (Fig. 11 *b*) like its effect on the general blood-pressure was definitely more prolonged than the corresponding constrictor effect of adrenaline (Fig. 11 *a*) on the same preparation.

The action of pitressin on the mesenteric veins is quite different from that of adrenaline. In Fig. 12 *a* it will be seen that a small dose of adrenaline produced in cats and dogs marked constriction of the mesenteric veins similar to its effect on the mesenteric arteries. The only effect seen after injection of pitressin (Fig. 12 *b*) was a small dilator effect.

The result then of the experiments with pitressin on the isolated mesenteric vessels is that, both in cats and dogs, it constricts the arteries and has, if any, a weak dilator effect on the veins.

(*b*) *Perfusion of the isolated livers of cats and dogs.*

Method. Livers isolated from cats and dogs were perfused simultaneously through the hepatic artery and the portal vein with the defibrinated blood of the same species. In the case of the cat the blood from three cats was used. The perfusion through the hepatic

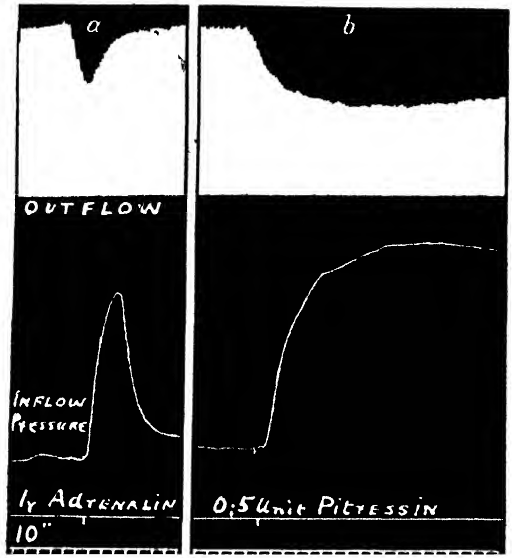

Fig. 11. Dog. Perfusion of isolated mesenteric arteries. (*a*) Injection of 1γ of adrenaline. (*b*) Injection of 0·5 unit of pitressin.

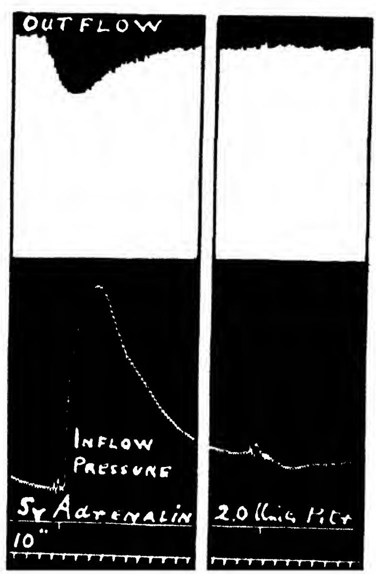

Fig. 12. Dog. Perfusion of isolated mesenteric veins. (*a*) Injection of 5γ of adrenaline. (*b*) Injection of 2·0 units of pitressin.

artery was performed by means of the Dale-Schuster pump, the portal vein was perfused from a blood reservoir by hydrostatic pressure. The records of the tracings beginning with the top one show: (1) outflow from the vena cava measured with the method described by Gaddum, (2) liver volume by plethysmograph, kept at constant temperature and connected to large bellows, (3) portal pressure recorded by means of a water manometer and a Brodie bellows, (4) arterial pressure in mm. Hg. Full details of the method are given in a paper by Bauer, Dale, Poulsson and Richards [1932].

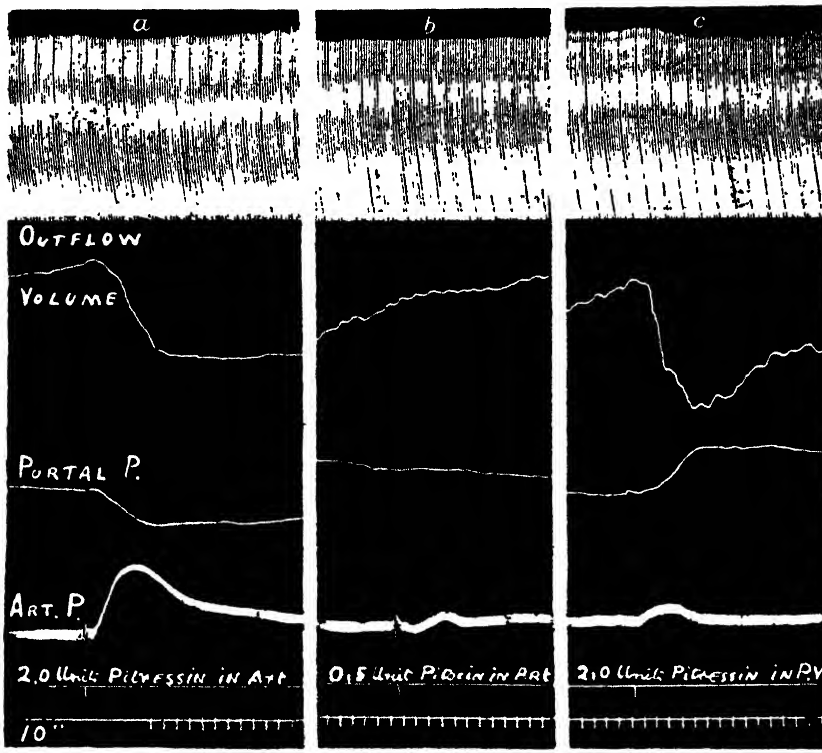

Fig. 13. Perfusion of cat's liver. (*a*) Arterial injection of 2·0 units of pitressin. (*b*) Arterial injection of 0·5 unit of pitocin. (*c*) Portal injection of 2·0 units of pitressin.

Results. Lampe and Méhes [1926] have performed experiments with pituitary extracts on the isolated liver of cats and dogs, Clark [1928] on the isolated dog's liver. In both cases the liver was perfused only through the portal vein, and pituitrin produced diminution of the liver volume and of the outflow from the vena cava, both of which effects were ascribed to constriction of the portal vessels. Adrenaline had a similar but stronger effect.

Cat's liver. The effects of pitressin and pitocin on the cat's liver are shown in Fig. 13. When pitressin is injected into the hepatic artery it produces a rise in the arterial pressure, a fall of portal pressure and a fall of volume (Fig. 13 *a*). These effects are probably due to the constriction of the branches of the artery, the fall of portal pressure being a direct mechanical result of the decrease in the rate of flow through the artery. The blood supply to the liver through the portal vein is thus somewhat increased and compensates almost completely for the decrease in arterial flow, so that only a just perceptible decrease in the total flow results, as shown by the slightly diminished outflow. When pitressin is injected into the portal vein, the portal pressure rises, presumably owing to constriction of the portal vessels (Fig. 13 *c*).

Pitocin has little or no effect on the circulation of the isolated liver of the cat (Fig. 13 *b*).

The fall in portal pressure and liver volume, seen after pituitrin in experiments on the whole cat, can thus be attributed to two causes: (1) selective constriction of the mesenteric arteries, leading to a diminution of the amount of blood flowing into the portal system, (2) constriction of the hepatic artery, leading to a decrease in the amount of blood reaching the liver by this route.

Dog's liver. As in the liver of the cat, the intra-arterial injection of pitressin produces a rise of arterial pressure (Fig. 14 *a*), but the accompanying fall of portal pressure and liver volume is more marked than in the cat, and the most striking difference between the two animals lies in the marked increase in the venous outflow which pitressin causes in the dog but not in the cat. The combination of these effects—fall in portal pressure and liver volume, and increase of outflow—can only be due to the relaxation of efferent vessels.

It is known that the dog's liver in contrast to that of the cat has a venous sluice mechanism [Mautner and Pick, 1923]. The efferent hepatic veins are constricted by small doses of histamine and, as has been found by Bauer, Dale, Poulsson and Richards [1932] and by Grab, Janssen and Rein [1929 *a*, *b*] relaxed by adrenaline. This sluice is apparently located near the openings of the hepatic veins into the vena cava, since extirpation of this region abolishes the effect. The corresponding effect of pituitary extract in my experiments similarly was found to be abolished by extirpation of the openings of the hepatic veins.

The present experiments thus show that the action of pituitary extracts on the vessels of the dog's liver is due to the relaxation of the efferent hepatic veins, the sluice veins of the dog's liver, and thus provide

an explanation for the fact that in the whole animal after an injection of pituitrin or pitressin portal pressure and liver volume fall, although the blood flow to the splanchnic area is increased (augmentation of intestinal volume). The pituitrin reaches the liver through the hepatic

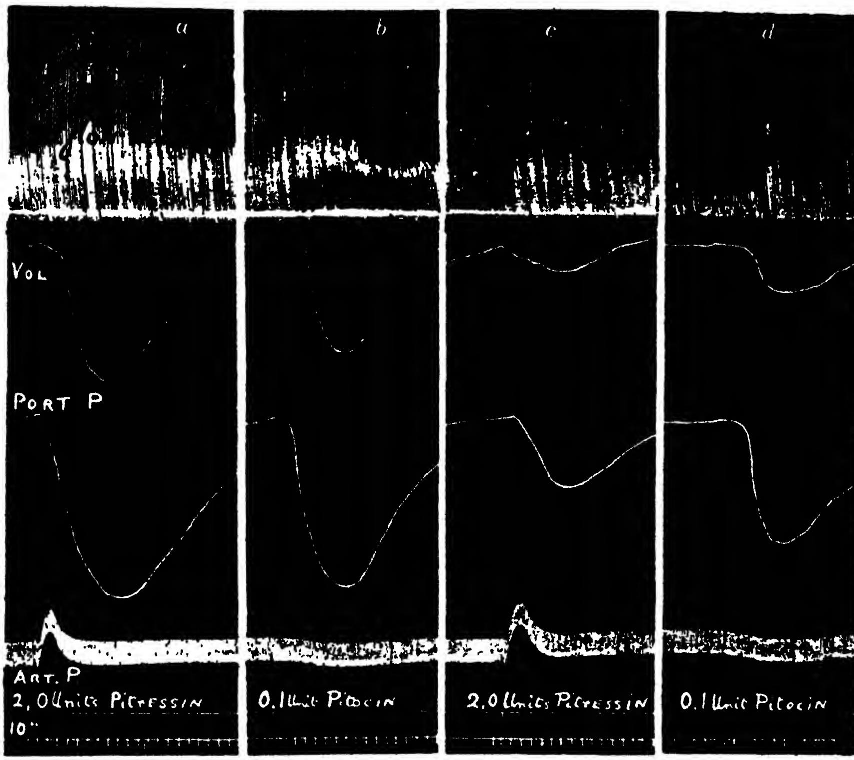

Fig. 14. Perfusion of dog's liver. (*a*) Arterial injection of 2·0 units of pitressin. (*b*) Arterial injection of 0·1 unit of pitocin. (*c*) Arterial injection of 2·0 units of pitressin. (*d*) Arterial injection of 0·1 unit of pitocin.

artery and relaxes the venous sluice, before the augmented blood flow to the splanchnic area can produce any rise in portal pressure. This relaxation is so marked that the total effect produced is a pure fall of portal pressure.

In the course of these investigations it was found that pituitrin, the complete extract of the posterior lobe, caused an even more pronounced increase of outflow from the dog's liver than pitressin, and the possi-

bility arose that this effect might be due not to the pressor principle itself, but to the oxytocic principle, which is always present in small quantities in the pitressin but which is of course present in larger quantities in the pituitrin. To test this point a preparation of the separated oxytocic principle, pitocin, was first compared with pitressin for its action on the guinea-pig's uterus. It was found that 20 units of pitressin, tested in this way, were equivalent to 1 unit of pitocin. If the suggestion mentioned above, that the action of pitressin on the venous outflow in the dog's liver is due to its content of oxytocic principle, were correct, pitocin should have the same effect as pitressin when given in 1/20th of the dose. That this is indeed the case is shown in Fig. 14: 2 units of pitressin have approximately the same effect as 0·1 unit of pitocin. These results show that the effect of pituitary extracts on the venous outflow of the dog's liver is due to some substance which is present in much higher proportions in pitocin than in pitressin, and there is no reason to doubt that this substance is the oxytocic principle itself.

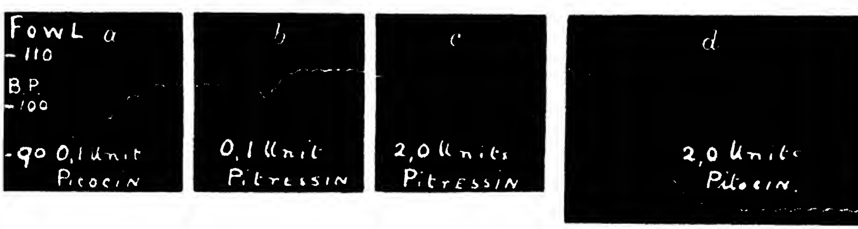

Fig. 15. Fowl. Urethane. Blood-pressure. Comparison between pitressin and pitocin.

Note on the action of posterior pituitary extracts on the blood-pressure of the fowl.

Support of the view that the dilator action of pituitary extracts (as seen on the mesenteric veins and the efferent hepatic veins of the dog's liver) is due to their content of oxytocic principle can be found in the result of some experiments on fowls. It is known that the blood-pressure of the bird, in contrast to that of the mammal, falls after the injection of posterior pituitary extracts [Paton and Watson, 1912; Hogben, 1925]. Gaddum [1928] and Morash and Gibbs [1929] found that this effect could be obtained with smaller doses of "oxytocin" than of "vasopressin."

Fig. 15 shows records from an experiment, in which a quantitative comparison was made between the action of pitressin and pitocin on

the blood-pressure of the fowl. From Fig. 15 *a* and *c* it can be seen that a twenty times smaller dose of pitocin (0·1 unit) than of pitressin (2 units) has approximately the same effect, and this effect can be obtained several times in the same experiment. Thus it seems conclusive that the fall of blood-pressure, as observed in birds after the injection of pituitary extracts, is caused by the oxytocic substance.

In Fig. 15 *b* a very small dose of pitressin (0·1 unit) has, after a slight dilator, a small pressor effect. The amount of oxytocic substance contained in the small dose of 0·1 unit of pitressin is apparently not big enough to overcome the pressor action which the entirely pure pressor principle of the posterior lobe probably would have also in the bird. Finally, Fig. 15 *d* shows the pronounced and very long-lasting dilator effect of a somewhat larger dose of pitocin (2 units).

Discussion.

A point needing a little further discussion is the question of the significance of the relaxation of the hepatic veins, as seen after the injection of pituitary extracts in the isolated liver of the dog, for the portal and the pulmonary circulation in the whole animal. It has been pointed out that the fall of portal pressure, which occurs in the whole animal in spite of the increased blood flow to the splanchnic area (increased intestinal volume), could be explained by assuming that the opening of the venous sluice in the dog's liver diminishes the resistance, against which the blood from the portal system flows into the vena cava. If in the whole animal, as in the isolated liver, the rate of outflow from the hepatic veins is thus augmented, this effect by itself would increase the flow of blood to the heart and lungs. The fall of pressure in the pulmonary artery and the diminution of lung volume, however, prove that, in fact, the total flow of blood to the lungs is diminished.

The blood flowing into the vena cava comes from two main sources: (1) from the splanchnic vessels through the portal vein, liver and hepatic veins, (2) from the limbs, body wall and the somatic area generally. In experiments in which limb and intestinal volumes were measured simultaneously it was found that the limb volume was diminished at the same time as the intestinal volume was augmented. The limb vessels of the dog, therefore, seem to be more sensitive to the constrictor action of pituitrin than the splanchnic vessels. Under these conditions the return of blood from the somatic area to the heart and lungs is diminished, the blood flow to the splanchnic area, in which the constrictor effect is

less powerful, undergoing a passive augmentation. The discharge of blood from the splanchnic area by the hepatic veins into the cava cannot, however, be increased to the same extent as that from the somatic area is diminished, since the total inflow to the right heart becomes less, causing a fall of the pulmonary arterial pressure. There must accordingly be some accumulation of blood in the splanchnic area, and we have seen that this occurs in the intestine, the volume of which shows a secondary increase. Owing to weakening of the resistance to hepatic outflow, the liver loses volume and the pressure falls in the portal vein. Since, as shown in perfusion experiments, the tone of mesenteric venules is not increased, but, on the contrary, somewhat diminished, the combined effect of swelling of the intestine and fall of portal pressure is still inadequately explained. It seems necessary to postulate a loss of tone in the intestinal capillaries to account for it.

SUMMARY.

The action of extracts of the posterior lobe of the pituitary gland on different parts of the circulation has been studied.

1. *Pulmonary circulation and heart.* Pituitary extracts have no action on the lung vessels. Either the complete extract (pituitrin) or the separated pressor fraction (pitressin) produces a rise of pressure in the pulmonary artery of the cat, which is secondary to an increase of coronary flow; they produce a fall of pulmonary arterial pressure in the rabbit and in the dog, which is due to a diminution of coronary flow. An additional cause of this fall in the dog is the accumulation of blood in the splanchnic area. The reason for the rise of pressure in the pulmonary artery in the heart-lung preparation of the dog is discussed.

2. *Portal circulation.* Pituitary extracts cause a fall of pressure in the portal vein both of the cat and the dog. In the cat this is due to selective constriction of the mesenteric arteries and to constriction of the hepatic artery. In the dog it is mainly caused by dilatation of the hepatic veins (opening of the venous "sluice" of the dog's liver). This dilator effect on the hepatic veins, produced by pituitrin and pitressin, is due to their content of oxytocic substance. (The fall of blood-pressure in the fowl after the injection of pituitary extracts is also due to the oxytocic principle.)

I wish to thank both the late Prof. W. E. Dixon and Sir Henry Dale, whose advice and criticism have been invaluable at different stages of this work.

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THE RESTING HEAT PRODUCTION OF NERVE.

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THE rate at which heat is liberated by a muscle at rest on a thermopile can be measured directly as a deflection on a galvanometer scale, provided that certain precautions are taken [see Hill, 1928 *a*]. These are (*a*) the use of instruments so well insulated that electrical leaks and disturbances do not occur; (*b*) the employment of a constant temperature bath of sufficient accuracy; (*c*) the avoidance of a vapour pressure difference between the muscle and the gas in the chamber. If this difference be not avoided [Hill and Kupalov, 1930] large errors may occur due to evaporation of, or condensation on, the muscle.

Similar methods can be used to measure the resting heat production of nerve. In the present work a differential nerve thermopile described by Downing and Hill [1929] was used together with a Downing moving magnet galvanometer (50 ohms resistance, 1 mm. = 4×10^{-10} amp. about). Foı the measurement of resting heat a differential thermopile has great advantages, since slight fluctuations which cannot be avoided in any bath, however well controlled, then produce less instability. A gas-regulated thermostat bath was employed, constant to about 0·002° C. under good conditions [see Hill, 1930]. To avoid a vapour pressure difference it was necessary to soak the nerve in Ringer's solution for a long time before readings were made. For this purpose the nerves must be firmly fixed to the thermopile so as not to be washed away by the fluid in the chamber.

In all experiments four sciatic nerves of Hungarian *Rana esc.* were used. After careful dissection they were mounted on the thermopile, each nerve running twice along the thermopile face, being wound round the two warming electrodes, one at each end. Ringer's solution was placed in the chamber (phosphate 10 mg. per 100 c.c., *p*H 7·2), the apparatus was placed in the bath and oxygen was bubbled through the solution to assist in temperature equalization, and to keep the nerve well oxygenated.

Nerves appear to take a long time to reach vapour pressure equilibrium with a surrounding solution, so that dissection usually occurred the evening before the experiment. The nerves therefore were soaked in Ringer's fluid all night—about 12 hours. When observations were to begin, the solution was removed and replaced by oxygen and in about 20 min. the first reading of the resting heat rate in oxygen was made. To obtain a good average the readings were continued for half an hour. The oxygen was then replaced by nitrogen, which had been purified by passing over hot copper and moistened by bubbling through water. This passed through the chamber rapidly for the first 4 min. and then more slowly. The slow passage of nitrogen continued throughout the period of asphyxia. This lasted for 3–5 hours; oxygen was then readmitted slowly so as not to cause a disturbance, and readings of the heat production during recovery from asphyxia were made.

At the end calibration was carried out with condenser discharges and a commutator, as recently described [Hill, 1931].

The temperature was about 20° C. throughout. Owing to the sensitivity of the arrangements used, variations of temperature in the bath, too small or too rapid to be read with a Beckmann thermometer graduated in 1/500° C., caused fluctuations in the readings of the resting heat rate, serious individually but capable of being avoided by taking an average over several minutes.

THE RESTING HEAT RATE OF NERVES IN OXYGEN.

Readings were taken every half minute for half an hour, and then the mean deflection for every 5 min. was calculated.

The following examples illustrate the procedure and the accuracy of the results.

Exp. of Feb. 12, 1932. Nerves dissected Feb. 11 at 7 p.m.; mounted on thermopile at 7.15 p.m. and put in Ringer's solution in bath at constant temperature. Feb. 12 at 10.35 a.m., Ringer's solution replaced by oxygen; at 10.55 a.m., readings begun.

Successive readings of the rate of heat production, each the mean for 5 min., were as follows, expressed in g. cm. of energy per g. of nerve per minute: 154, 152, 161, 148, 168, 157, 157, 151; mean 155.

Exp. of Feb. 15, 1932. Nerves dissected and put in Ringer's solution on thermopile in bath, Feb. 14 at 7 p.m. Next morning at 11.10 a.m. solution replaced by oxygen; at 11.53 a.m. readings begun. Mean for 30 min.: 224 g. cm. of energy per g. of nerve per minute. Mean variation of the 5 min. averages from the mean value: 2 p.c.

The results of eight experiments of the resting heat rate of nerves in oxygen are as follows: the total weight of the four nerves employed was

about 180 mg.: 155, 116, 172, 134, 186, 155, 224, 224; mean 176 g. cm. of energy per g. of nerve per min., or $4 \cdot 14 \times 10^{-3}$ cal. per g. per min.

The mean value agrees closely with that for muscle at the same temperature. Hill [1928 *a*, p. 139] gives 160 g. cm. per g. per min. as a "reasonable minimum" for 20° C. For nerves at rest at 14° C. Gerard [1927] found a mean oxygen consumption of $2 \cdot 7 \times 10^{-4}$ c.c. per g. per min. Extrapolating by means of a temperature coefficient of 2·5 for 10° C., the value at 20° C. should be $4 \cdot 7 \times 10^{-4}$ c.c. per g. per min. Taking 5 cal. as equivalent to 1 c.c. of oxygen, Gerard's extrapolated value is equivalent to $2 \cdot 35 \times 10^{-3}$ cal. per g. per min., or about 100 g. cm. per g. per min. According to Fenn [1927] at 22° C. the resting oxygen consumption of frogs' sciatic nerve is $1 \cdot 23 \times 10^{-3}$ c.c. per g. per min., which, extrapolated to 20° C. by the same temperature coefficient, would be $1 \cdot 03 \times 10^{-3}$ c.c. per g. per min. This is equivalent to $5 \cdot 15 \times 10^{-3}$ cal. or 220 g. cm. per g. per min. The mean value found in the present experiments lies almost exactly half-way between those calculated from the oxygen measurements of Gerard and Fenn.

Anaerobic resting heat production.

When oxygen was replaced by nitrogen, within 15 min. the rate of heat production began slowly to decrease. After 40 min. it had fallen to 75 p.c. of its initial value; at 1½ hours to 50 p.c. and at 2½ to 3½ hours to 20–25 p.c. After reaching 20–25 p.c. of its original level the heat rate ceased to fall and became constant apparently indefinitely.

Fig 1 shows the behaviour of the heat rate during anaerobiosis. It was not possible to determine how long it remained at the minimum level. Four to four and a half hours after removing the Ringer's solution there might occur a slow increase of heat rate overshadowing the previous fall. This may have been due either to a decrease of vapour pressure caused by anaerobic breakdown or to some genuine heat production resulting from the injurious effect of long oxygen want. Observation showed at any rate that no significant increase occurred for 30–40 min. after the heat rate had fallen to one-quarter or one-fifth of its original level.

Table I gives in g. cm. per g. per min. the minimum heat rate of a resting nerve deprived of oxygen for 2–3 hours. For comparison the initial heat rate in oxygen also is given, and in the last column the former expressed as a percentage of the latter.

In order to decide to what degree the failure of the resting heat production depends upon the amount of oxygen initially present in the

TABLE I.

Date	Initially in O_2	Means in successive 5 min. in N_2	Mean of all in N_2	In N_2 as p.c. of initial in O_2
20. i. 32	172	50·0, 47·6, 49·9, 52·0	49·9	28·3
30. i. 32	134	—	29·1	21·7
2. ii. 32	224	42·5, 55·0, 44·4, 44·4, 49·3	47·8	21·0
12. ii. 32	186	47·3, 47·0, 47·6, 47·8	47·3	25·0
15. ii. 32	224	42·5, 55·0, 44·4, 44·4, 49·3	47·8	21·0

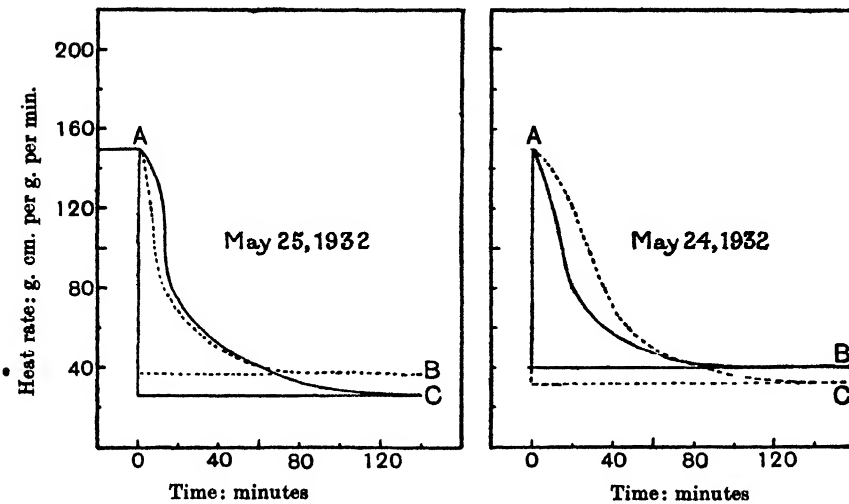

Fig. 1. Resting heat production of nerve in nitrogen at 21° C. after oxygen or air.
Left: ...... *AB*, First asphyxiation after air.
—— *AC*, second asphyxiation after recovery in O_2.
Right: ...... *AC*, first asphyxiation after O_2.
—— *AB*, second asphyxiation after recovery in air.

environment, or in the nerve itself, four experiments were done in air instead of oxygen. All the conditions were the same, but instead of oxygen, air was bubbled through the Ringer's solution during the night. Then the resting heat rate, first in air and after that during asphyxiation, was read. The results are given in Table II.

TABLE II.

Date	Heat initially in air g. cm./g. min.	Mean minimum value in N_2 g. cm./g. min.	In N_2 as p.c. of initial in air
18. v. 32	179	37·6	19·3
20. v. 32	238	59·0	23·5
23. v. 32	180	37·4	20·7
25. v. 32	198	51·1	25·7
Mean	198·8	45·5	22·9

Comparing Tables I and II, we see that the resting heat rate and the minimum steady level reached during asphyxia are the same in both cases; the time, however, for reaching the minimum level for the nerve initially in air is less than for that initially in oxygen. If the latter reaches this level in about 2½ hours, the former reaches it in about 1½ hours.

Since the experiments in air were done considerably later than those in oxygen and partly with a new stock of frogs, several experiments were made with double asphyxiation of the same nerve—one after air, the second after recovery in oxygen. The order of the experiments was as follows:

The nerves were kept overnight in Ringer's solution saturated with air, then asphyxiated. After 2–2½ hours in nitrogen the nerves were recovered by oxygen. After being in oxygen during 2–2½ hours the second asphyxiation took place and the rate of heat production was observed. Another series of experiments was done in the opposite order: the first asphyxiation was after oxygen overnight and the second one after 2–2½ hours recovery in air.

Fig. 1 shows the results of two experiments. Here, for comparison, all the heat rates are reduced to the same initial level. The dotted line shows the first asphyxiation, the full line the second. On May 25 the first asphyxiation was after air, on May 24 after oxygen. The full lines show the heat rate during asphyxiation after 2½ hours' recovery in oxygen (May 25) and in air (May 24).

In both cases the rate of decrease of the resting rate of nerves during asphyxiation is slightly greater for nerves initially in air, whether this asphyxiation was the first or the second of an experiment.

The mean value of the anaerobic heat rate was 42·4 g. cm. per g. per min. This is only two-thirds of the value (65) given by Hill [1928 *a*, p. 141] for muscle in nitrogen at 20° C. Hill's value was confirmed in two experiments made specially on muscle, which gave 72·5, 55·9, mean 64·2. These values were respectively 45 p.c. and 56 p.c., mean 50 p.c., of the heat rate initially in oxygen. The usual ratio of 1 to 2 was found, in striking contrast with the case of nerve, where the ratio is 1 to 4 or 1 to 5.

There is a further difference between muscle and nerve. In muscle the fall of resting heat rate is complete in 30–60 min. after oxygen is replaced by nitrogen; in nerve the diminishing heat rate continues for not less than 2½ hours. Moreover in muscle, as is well known, in recovery after asphyxia the amount of excess heat is about equal to the amount of heat missed during the period of oxygen want. In nerve, as we shall see, the excess is only a small part of the heat missed.

THE RECOVERY HEAT PRODUCTION AFTER ASPHYXIA.

Fig. 2 shows the behaviour of asphyxiated nerve when oxygen is readmitted. The heat rate begins to increase immediately; in 5 min. it reaches the original level and in 10–15 min. it may be 50–60 p.c. above the original level. During the next 15 min. a rapid decrease occurs which

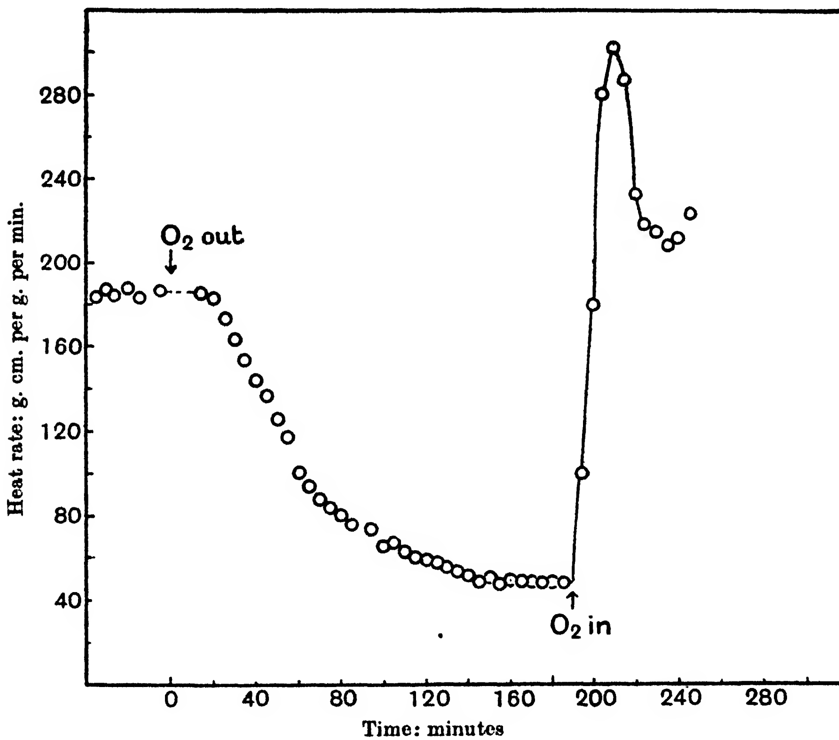

Fig. 2. Rate of heat production of nerve in oxygen and in nitrogen at 20° C. Oxygen removed at the moment shown by the first arrow and reintroduced at the moment shown by the second arrow.

afterwards becomes slower until a steady level is reached. In one experiment only (Fig. 3) was the final level after recovery from asphyxia the same as the original level in oxygen. Generally after decreasing to 115–120 p.c. of its level, the heat rate began slowly to rise again, due to some unknown cause, perhaps to a harmful effect of long oxygen want.

The maximum heat rate observed during recovery had a mean value of about 7×10^{-3} cal. per g. per min., which is about seven times the minimum heat rate in nitrogen.

The most striking thing about these results is the smallness of the recovery heat in comparison with the heat missed in nitrogen. Let us calculate the amounts.

Exp. of Jan. 7, 1932.

Mean heat rate of nerve in oxygen	155 g. cm. per g. per min.	
„ „ nitrogen	79·4	„

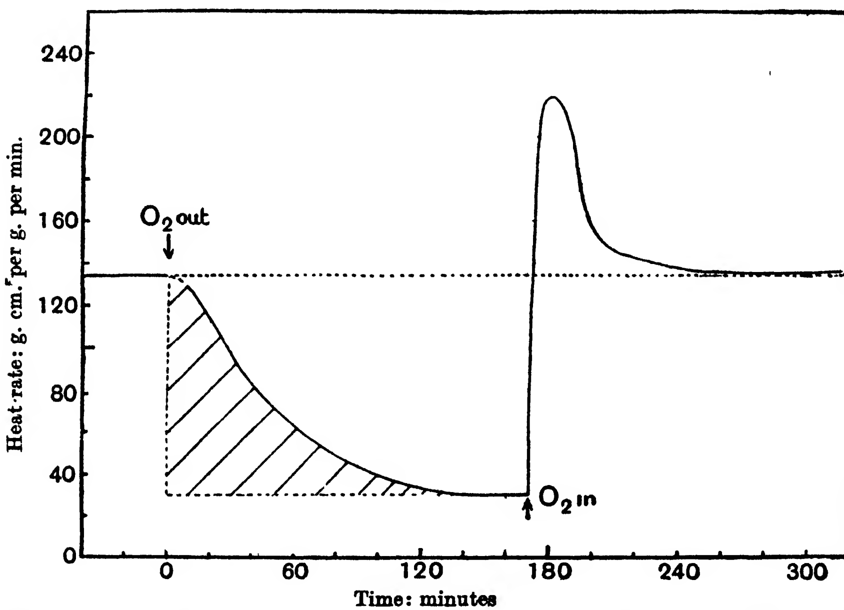

Fig. 3. Resting heat production of nerve in oxygen, in nitrogen and during recovery at 20° C. Note the return to the original level of recovery. The shaded area represents heat assumed to be derived from oxidation after molecular oxygen has been swept away.

Total heat in nitrogen during the whole period of asphyxiation (3 hours 12 min.) = 15230 g. cm. per g.

Total resting heat in oxygen for same period = 29800 g. cm. per g.
Heat (missed) in nitrogen = 14570 g. cm. per g.

Recovery heat after oxygen readmitted:

Mean heat rate 2 hours after	174 g. cm. per g. per min.
Total heat during recovery (2 hours)	20800 g. cm. per g.
Excess heat for this period	20800 − 18600 = 2200 g. cm. per g.

Thus we have:

Heat missed in nitrogen	14570 g. cm. per g.
Excess heat during recovery	2200 g. cm. per g

It is interesting to compare the excess heat of recovery found here with the excess oxygen found by Fenn [1930]. Fenn found that after a period of oxygen want nerves consumed an extra amount of oxygen which, however, was less than one-quarter and frequently less than one-tenth of the oxygen missed during the anaerobic period. The phenomenon found here is analogous to that found by Fenn. The extra oxygen and the extra heat when oxygen is admitted after asphyxia are presumably related.

The relative smallness of the recovery heat and the recovery oxygen may be related to the fact found by Gerard and Meyerhof [1927] that lactic acid accumulates in nerve during oxygen deprival but is not resynthesized and is scarcely oxidized when oxygen is readmitted.

Discussion.

In view of the above results two questions arise: (1) from what source does the anaerobic resting heat rate of nerve arise, and (2) to what processes can the extra heat production during recovery from asphyxia be attributed?

In the anaerobic heat production there are two phases, their heat being derived presumably from different sources. The first corresponds to the falling heat rate (from 100 to 25 p.c.) during the first 2–3 hours, the second corresponds to the "steady state" reached in 2 or 3 hours and lasting probably for many, apart from slight disturbing factors.

The heat production of the first phase is probably a resultant of two processes: (*a*) the using up of the oxygen store and (*b*) the anaerobic breakdown of some substances, probably chiefly of carbohydrate to lactic acid.

That some oxidative processes take place during anaerobiosis is indicated by Gerard's discovery [1927] that during long asphyxia a nerve produces (not merely gives off) about 10 p.c. as much carbon dioxide as during the same time in oxygen. The question is what kind of oxygen it can be—whether a true "oxygen store," the existence of which in the nerve is suggested by various authors, or whether molecular oxygen initially dissolved in the nerve tissue, which does not escape by diffusion.

Let us calculate the amount of heat production due to this "oxidative" process. We can do it assuming that during asphyxia all the heat above the minimum steady level (equal to 20–25 p.c. of the initial level) is derived from this source. In Fig. 3 the shaded area represents the heat assumed to be so derived.

In the experiments of Jan. 7 and Jan. 30 the "oxidative" heat calculated in this way during asphyxia had the values 4240 and 2430 g. cm. per g., and in all the experiments performed the mean value was 3120 g. cm. per g. This would be enough to last at the full resting heat rate in oxygen for about 20 min.

The total amount of oxygen corresponding to it can be calculated, assuming again that 5 cal. are equivalent to 1 c.c. of oxygen: 3120 g. cm. per g. is then equivalent to 0·0146 c.c. of oxygen per g. This is quite a small amount when we remember that water saturated with oxygen at 20° contains 0·031 c.c. of oxygen per g., so that nerves containing 80 p.c. of water would hold 0·025 c.c. of oxygen per g. in simple solution.

In what way will this "oxidative" heat be changed in the case of nerves initially in air? Since the solubility of oxygen depends upon its partial pressure, and taking account of the nerves' metabolism, the amount of oxygen dissolved will be equivalent to about 0·004 c.c. per g. If dissolved oxygen were the cause of the "oxidative" heat in question, this would be reduced to less than one-fifth by using air. If the heat were due to a true oxygen store of some kind it would be nearly unchanged.

Calculated as above, the "oxidative" heat for the experiments of May 18, 20, 23 and 25 for the nerve initially kept in air are: 1945, 1470, 1909 and 2400 g. cm. per g. respectively; mean 1931 g. cm. per g. The amount of oxygen corresponding to 1931 g. cm. is 0·0091 c.c. per g. This is nearly twice as great as the amount which could initially have been dissolved in the water of the nerve.

What other source of heat could be suggested for the first stage of asphyxiation? One possible source is the breakdown of phosphagen to creatine and phosphate. According to Gerard and Tupikow [1930] labile phosphorus amounting to about 4 mg./100 g. is broken down in asphyxia. According to Hill's calculation [1932] this would liberate $1{\cdot}44 \times 10^{-2}$ cal. per g. of nerve, which at 10^{-3} cal. per g. per min. would last for 14 min. The quantity is so small that although it may have something to do with the first falling phase, it cannot explain it altogether.

It is difficult to say at present in what way the greater rate of asphyxiation, for the nerve kept previously in air, can be explained. It may be that the diffusion constant for the nerve is much smaller than is generally assumed, and more of the oxygen dissolved in the tissue could be used during asphyxiation before it diffused away; if so, however, owing to metabolism, the amount dissolved in the resting nerve would be

less; or it may be that the nerve produces less of the oxygen store if it is kept in air.

The question requires further investigation. At any rate, however, even if the diffusion constant for the nerve is so abnormally low that nearly all the oxygen dissolved in the tissue can be used by the nerve during asphyxiation, an amount of "oxidative" heat remains, which cannot be explained without the supposition that the nerve uses oxygen in other than molecular form.

Returning now to the second phase, this probably involves a breakdown of glycogen which is known to occur at an increasing rate, as shown by Gerard and Meyerhof [1927]. They found that the rate of lactic acid formation is slow during the first hour of oxygen want; about 3 hours from the beginning of asphyxia it occurs at a steady rate. It is interesting to calculate the amount of lactic acid to which the minimum heat rate of the asphyxiated nerve would correspond. The calculation suggests that *all* the heat production of this second phase is due to lactic acid formation. Taking (as above) 10^{-3} cal. per g. per min. as the minimum steady heat rate in asphyxia at 20° C., and assuming that the liberation and neutralization of 1 g. of lactic acid would lead to the production of 300 cal., the calculated lactic acid formation is $3 \cdot 3 \times 10^{-6}$ g. per g. of nerve per min., or 2×10^{-4} g. per g. per hour. Gerard and Meyerhof, working at 15° C., found over long periods of asphyxia a lactic acid formation of $1 \cdot 25 \times 10^{-4}$ g. per g. per hour. Applying a temperature coefficient of 2·5 to bring their results to 20° C. we get almost exactly 2×10^{-4} g. per g. per hour, corresponding to the number just calculated from the minimum heat in the present experiment.

When oxygen is readmitted to the asphyxiated nerve the excess heat is 2000–3000 g. cm. per g. This is several times as great as the heat (about 600 g. cm. per g.) liberated, as calculated above, by phosphagen breakdown. It may in part represent waste energy in the process of phosphagen resynthesis under the influence of oxidation. There is no indication at present of any other source of, or function for, this heat.

SUMMARY.

1. The rate of resting heat production of frogs' nerve at 20° C. has been studied in oxygen and during oxygen lack.

2. In oxygen it is 176 g. cm. or $4 \cdot 14 \times 10^{-3}$ cal. per g. of nerve per min.

3. During asphyxiation the resting heat rate slowly diminishes, falling in 3–3½ hours to 20 or 25 p.c. of its initial value.

4. The mean value of this minimum heat rate during asphyxiation was 42·5 g. cm. or 10^{-3} cal. per g. of nerve per min.

5. When oxygen is readmitted extra heat is produced which is (after 3–4 hours anaerobiosis) between 15 and 20 p.c. of the heat missed during oxygen want.

6. The resting heat rate in oxygen corresponds to the oxygen consumption measured by other observers, and the minimum resting heat rate in nitrogen to the lactic acid formation observed by Gerard and Meyerhof. The smallness of the recovery heat after asphyxia agrees with Fenn's observation of the smallness of the recovery oxygen under the same conditions.

I am deeply indebted to Prof. A. V. Hill for his suggestion of the present work and for his helpful advice and criticism. My sincere thanks are due also to Mr J. L. Parkinson and Mr T. P. Feng for their willing assistance throughout the investigation.

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THE FUNCTION OF THE ADRENAL MEDULLA.

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We have reported in a previous note[2], that under certain conditions extracts of the adrenal medulla show an activity which cannot be explained by the adrenaline present. This activity was apparently due to a substance similar to adrenaline, but more potent than the latter. Till a chemical name can be substituted we propose to call this substance "Novadrenine."

It is not the object of the present paper to give an exhaustive analysis of the physiological activity of novadrenine. We only want to give fuller evidence of the existence of such a substance, and to call the attention of research workers to this field of investigation. The present authors feel incompetent to give a definite physiological analysis, and are greatly hampered in their work by the lack of material.

The existence of novadrenine is based on the observation, that under certain conditions the potency of adrenal extracts is 10–15 times higher than would correspond to their adrenaline content, estimated chemically by a colorimetric method. This high activity was only observed if the glands were excised soon after death and cooled carefully. In a great number of experiments the adrenaline content of the ox medulla was estimated colorimetrically and was found to lie between 1–2 mg. per g. of tissue, whether the extract showed a high physiological activity or not. In active extracts the physiological activity corresponded to an adrenaline content of 15–30 mg. of adrenaline per g. of medulla, a difference which could hardly be explained by an experimental error. If this latter value were correct, the gland would contain 10–15 p.c. of adrenaline by dry weight, which is impossible. It was also frequently observed that during chemical manipulations the excess activity of the extracts suddenly disappeared, the values obtained by chemical analysis and physiological estimation being then in close agreement.

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[2] *Nature*, April 9, 1932.

It was thus supposed that the potency of our extracts was partly due to the presence of a substance more potent than adrenaline itself. Whether this substance, novadrenine, is built up from adrenaline under certain conditions, or adrenaline is a cleavage product of the former, cannot be stated at present.

Owing to the lack of a specific chemical reaction for novadrenine, it cannot be stated in what proportion our active extracts contained adrenaline and novadrenine. Consequently only a minimum estimate of the relative activity can be given. If, for instance, our extract shows a potency ten times as great as that corresponding to the adrenaline content, we may conclude that novadrenine is at least ten times as active as adrenaline. This would be the case, if our extract contained only novadrenine, and if adrenaline and novadrenine gave identical colour reactions. If, however, only 1 p.c. of the adrenaline was present as novadrenine, the latter would be a thousand times as potent as adrenaline.

It was reported in our preliminary note, that, when given in small doses to the cat, novadrenine does not cause a fall of blood-pressure, and that in this respect its action differs qualitatively from that of adrenaline. This observation, however, could not be verified later, and there is no reaction known at present in which novadrenine behaves qualitatively in a different way from adrenaline.

METHODS.

Material. All our experiments were made with ox suprarenal glands. The glands were excised as soon as possible after death and placed on ice and transported to the laboratory. Here they were chilled at − 20° C. The chilled glands were cut open, and medulla and cortex were separated. The medullary tissue was minced in a fine mincer with holes of 1 mm. diameter. The pulp was suspended in a ½ p.c. solution of trichloroacetic acid, 2 c.c. being used for every gram of the pulp. The suspension was rapidly heated to 80° C. and rapidly cooled again. The extract was filtered and the clear solution was kept in the refrigerator at − 20° C. Extracts were kept here for more than a week without apparent loss of activity.

Colorimetric estimation. The quantity of adrenaline present was estimated by the following method. The extract was neutralized with a small excess of sodium bicarbonate. Lugol solution was added, till the brownish red colour indicated an excess of iodine. The excess of iodine was removed by 0·1 *N* sodium thiosulphate, leaving a pink colour, which

was stable for a minute or so, and could be compared with the colour of a known solution of adrenaline.

This colour reaction was controlled with a number of generally used colour reactions, active extracts being compared with adrenaline solutions. We used the reaction with mercury chloride and the persulphate test of Ewins, both tests being based on the coloured oxidation product of adrenaline. We tested the ferric chloride reaction, in which the *o*-dihydroxy groups are estimated. The Folin-Dennis test was also used, which is based on the reducing power of adrenaline. All these tests gave identical results with our colour test. Only the Folin-Dennis test

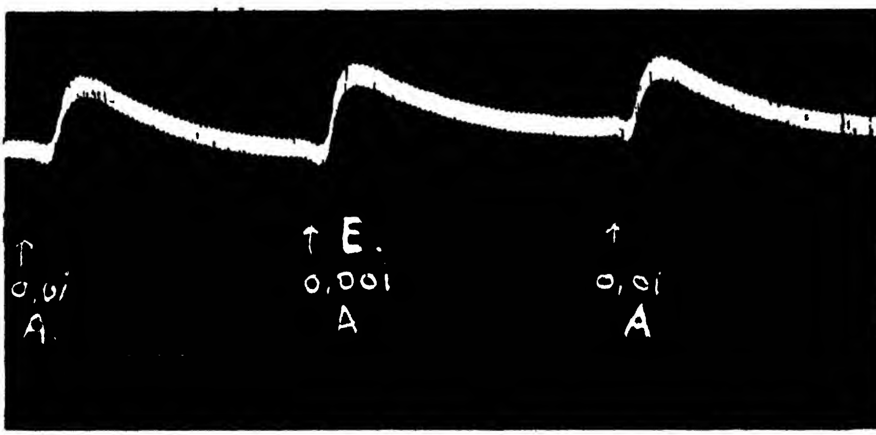

Fig. 1.

gave higher values with extracts containing hexuronic acid, which acid readily reacts with the reagent. Adrenaline added to our extracts was readily indicated by our reagent.

Other methods of extraction, as by cold 5 p.c. trichloroacetic, hot 0·1 p.c. acetic, or methyl-alcohol showed no advantage. The first was as good as the hot ½ p.c. trichloroacetic, the latter two were distinctly inferior.

Experiments.

If small doses of adrenaline are compared in the decapitated cat with active extracts containing colorimetrically the same amount of adrenaline, a much stronger pressor response will be given by the latter. If the dose of extract is gradually decreased, the dose of adrenaline being kept constant, it will be found that one-tenth to one-fifteenth of the dose of

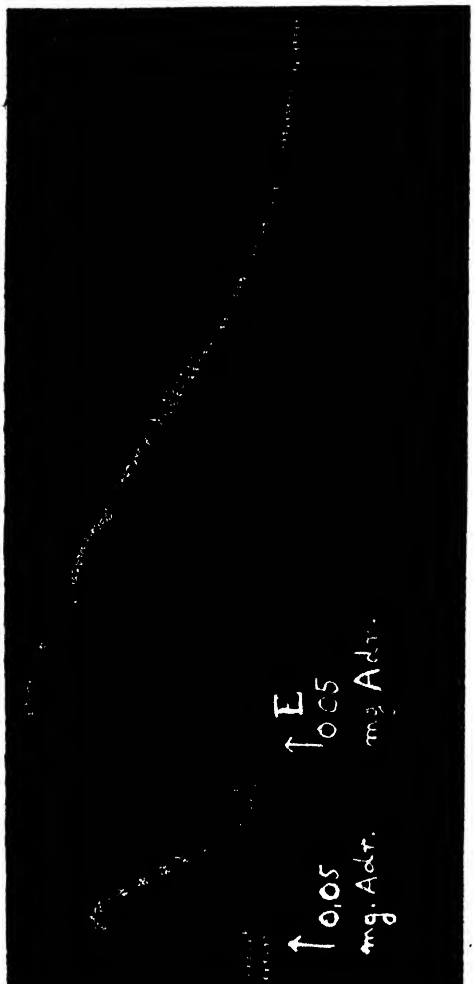

Fig. 2.

Fig. 3.

extract will give a response equal to that with adrenaline. The same is the case in cats after decerebration or under ether anæsthesia, provided the animal does not give a depressor response, which makes the results incomparable.

Fig. 1 illustrates the point. In this experiment the action of 0·01 mg. of natural adrenaline is compared with that of a diluted extract, containing 0·001 mg. of adrenaline, as determined colorimetrically, in a cat under ether. (Time tracing, 3 sec.)

In Fig. 2 the action of 0·05 mg. of adrenaline is compared in a decerebrated cat with an extract, containing the same amount of adrenaline, by colorimetric estimation.

If the effects of big doses of adrenaline, producing a rise of blood-pressure to the maximal height, are compared with those of an extract giving an identical colour reaction, the response to the latter will show a much longer duration. This is illustrated in Fig. 3 in the rabbit (ether anæsthesia).

If active extracts are compared with adrenaline, for activity on an isolated loop of rabbit's intestine, the inhibitor action of the extract will be found much stronger. Equally strong inhibitor actions are produced by adrenaline and by the extract, when the latter contains 10–15 times less adrenaline as measured colorimetrically.

In the Trendelenburg frog preparation a similar difference is found in the vaso-constrictor activity.

Summary.

The fresh adrenal medulla contains under certain conditions a substance having a more potent pressor action on the blood-pressure, and a more potent inhibitor action on the isolated gut than adrenaline. The substance is called "novadrenine." The results are discussed and supported by figures.

We are indebted to Sir H. H. Dale for personal mention of some observations of his own, and for helpful criticism of our results.

This research was sponsored by the Ella Sachs Plotz Foundation.

NOTE ON THE PREPARATION OF CRYSTALLINE ADRENALINE.

By the following method this can be done without the use of alkali. It is hoped that the separation of crystalline novadrenine, in the hands of workers in command of suitable material, may thus become possible.

The trichloroacetic extract (300 c.c.) is neutralized with NaOH and concentrated *in vacuo* at a low temperature to a small volume (3–5 c.c.). Gradually about 50 c.c. of methyl-alcohol are added, the inactive precipitate separated, and the fluid concentrated again *in vacuo* to an oily liquid. To this, 5 c.c. of methyl-alcohol and then 15 c.c. of acetone are added. The oily precipitate is separated on the centrifuge. To this precipitate again 5 c.c. of methyl-alcohol are added. After centrifuging off the inactive precipitate, 15 c.c. of acetone are added to the fluid. Again the precipitate is separated and discarded. To the two combined acetone-alcohol solutions a solution of ferrous chloride in aqueous acetone is added. This ferrous reagent is prepared by adding 9 parts of acetone to a saturated watery solution of the salt. After the precipitated excess of the salt has settled, the supernatant clear liquid is used.

On addition of the ferrous reagent a deep purple-blue precipitate separates consisting of the ferrous complex of adrenaline. This precipitate forms only at neutral reaction. On addition of the ferrous reagent the solution turns acid and must be neutralized with an acetone solution of ammonia (1 part of strong watery solution of ammonia and 4 parts of acetone). The ferrous reagent and ammonia are alternately added till, at neutral reaction, no more coloured precipitate is formed in the presence of iron. The precipitate leaves a slightly coloured blue liquid behind. The precipitate is separated on a Büchner funnel, suspended in little water (15 c.c.), and a vigorous current of H_2S is passed through. The fluid is quickly filtered on a Büchner. Very soon the crystallization of adrenaline begins, which is completed by cooling in an atmosphere of nitrogen.

The mother liquor contains only about 0·2 mg. of adrenaline per c.c. The yield is nearly 2 mg. of adrenaline per gram of medulla, which is not far from the total adrenaline content of the gland. The method can be applied with the same result to extracts of the whole gland.

If the preparation of novadrenine is attempted, possibly low temperatures should be applied for the concentration of the extract. Novadrenine is also carried down by the ferrous reagent, and can be liberated by H_2S without losing its activity.

THE SUPERNORMAL PHASE IN MUSCULAR CONTRACTION.

By TAKEO KAMADA.

(*From the Department of Physiology and Biochemistry, University College, London.*)

The isometric response of a muscle to a single shock can be measured, not only by the maximum tension developed, but by the area of the tension-time curve. The importance of tension-time is that it is the basis

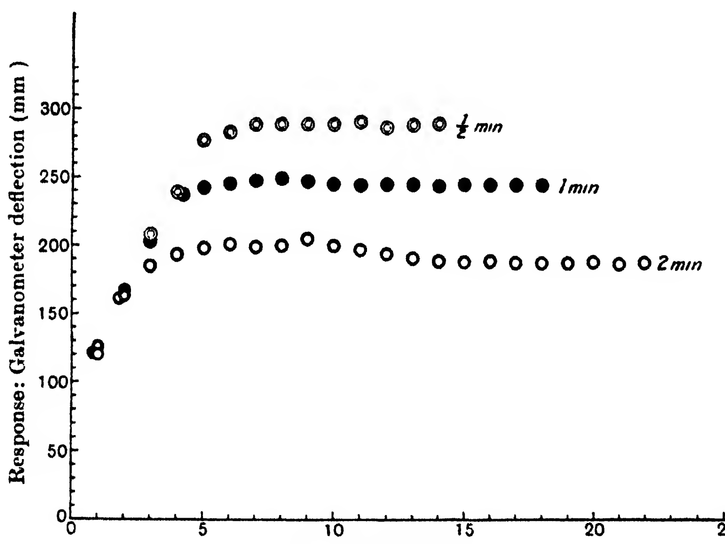

Fig. 1. Attainment of steady state during regular series of twitches. Gastrocnemius at 0° C. in O_2 Ringer. Horizontally, order of twitch in regular series; vertically, response (tension-time) as galvanometer deflection (mm.).

The three series were made in order: 2 min. intervals, 1 min. intervals, ½ min. intervals. After the first two series a long rest was given.

of the maintained contraction: the "economy" of a tetanus depends upon the "area" of a single twitch. The method described below allows the integration necessary for determining the tension-time to be performed instrumentally and the results to be read as the ballistic deflection of a galvanometer.

Recorded as tension-time the response of a muscle to a single shock shows a striking supernormal phase [Hartree and Hill, 1921] in the sense that at a suitable interval the response to a second shock is greater than that to the first one. Other aspects of this supernormal state have been referred to recently by Hill [1931 *a*, p. 296] and by Rushton [1932, p. 244]. In the present research its characteristics have been further investigated.

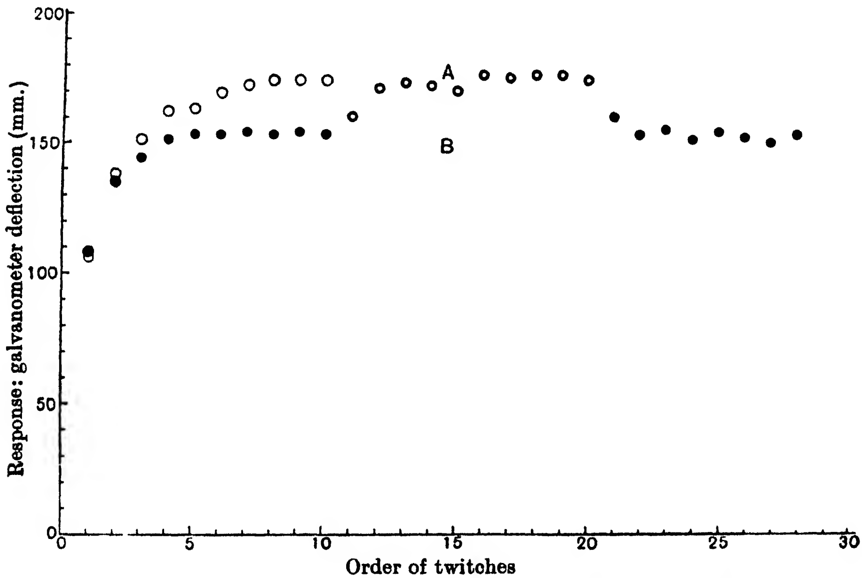

Fig. 2. Change in steady state due to change of interval between twitches. Gastrocnemius at 0° C. in O_2 Ringer. Horizontally, order of twitch in regular series; vertically, response (tension-time) as galvanometer deflection (mm.).

The series with 2 min. intervals (solid circles) occurred first. After the establishment of a steady state (level *B*) the interval was diminished to 1 min. (double circles). When the new steady state (level *A*) was established the interval was changed back to 2 min. and a third steady state was found at the same level *B* as before (solid circles). After about one hour's rest the muscle was stimulated again in a regular series at 1 min. intervals (hollow circles); the steady state was now reached at level *A*.

If a regular series of shocks be given to a muscle at not too high a rate the tension-time response (hereafter referred to as the response) increases until it reaches (apart from the onset of fatigue) a stationary level (see Fig. 1). Increasing the interval then causes a fall, decreasing the interval a rise in the level (see Fig. 2). If at any moment after one of the shocks of such a series an extra shock be interpolated, the response is greater or less according to the relations described below.

METHOD.

A differential cuprous oxide photoelectric cell was employed, as suggested by Hill [1931 *b*]. It was joined to a slow sensitive moving coil galvanometer employed ballistically. The gastrocnemius of a small Dutch frog (*R. esc.*) was connected by a wire to an isometric spring myograph, from the mirror of which a strong beam of light was reflected on to the middle of the cell. When the muscle contracted, the beam of light moved and generated a current in the cell. The amount of current so generated and recorded by the galvanometer was proportional to the tension-time.

The muscle was stimulated by single super-maximal induction shocks through its nerve. Two Harvard coils placed at right angles on the table were adjusted to give identical shocks, their secondaries being arranged in series with the electrodes. The time interval was regulated by a Lucas revolving contact breaker, with one key in the primary of each coil. When many successive shocks were given the cam contact breaker described by Gerard, Hill and Zotterman [1927] was used with a single coil. The preparation was immersed in phosphate Ringer's solution (pH 7·2), oxygen or nitrogen being passed continuously.

RESULTS.

When a muscle was stimulated at regular intervals with single shocks, the response increased gradually to a constant value (Fig. 1). During this "steady state" the response depends on the interval between shocks, being greater with shorter intervals. Usually the steady state was stable, except in the absence of oxygen, when fatigue set in. The supernormal phase was tested after the establishment of the steady state by interpolating an extra shock. The muscle then had to contract twice in one interval, so that the next regular shock gave a slightly greater response. Continuing, however, with the regular series the steady condition was re-established.

Following this routine, the supernormal effect at the second of two stimuli was determined with various intervals. Two different stages are found: (*a*) the relatively refractory phase, in which the second response is less than the first; (*b*) the supernormal phase, lasting up to the next regular shock, in which the second response is the greater. A third, but artificial, subnormal phase is then found in which, owing to the necessary omission of the next regular shock if an interval greater than that of the series is to be employed, the response is less than in the series.

TABLE I. Gastrocnemius at 0° C. in O_2 Ringer: regular series at 1 min. intervals. The supernormal value was obtained by subtracting double the regular response from the summated response (or when possible the regular response from that to the interpolated shock). With intervals more than 2 sec. the galvanometer was short-circuited during the first response: then the short-circuit was opened, so as to obtain the second response only.

Interval between shocks (sec.):											
0·1	0·75	1·25	1·75	7	20	30	45	60	75	90	120
Supernormal effect (p.c.):											
−63	+36	+34	+34	+35	+15	+13	+4	0	−2	−7	−10

When, during a regular series of shocks, a single one is replaced by a group in rapid succession, the extra activity disturbs the steady state and the next response in the regular series is enhanced.

TABLE II. Gastrocnemius at 0° C. in O_2 Ringer: regular series at 1 min. intervals. Excess of response at next regular shock as the result of extra activity immediately following a shock of the regular series. (Extra activity represented by number of extra shocks at 0·5 sec. intervals.)

Extra shocks	1	2	3	6	9	14
Excess response (p.c.)	5	8	11	15	18	20

The supernormal phase following a single shock can clearly be regarded as the first step in the establishment of a new steady state. This is true even of as short an interval as 0·5 sec. at 9° C. In such a case each shock is applied during and not after the previous contraction, and the total effect of a group of shocks must be read as a whole. With the longer groups the galvanometer had to be employed beyond the range of its ballistic proportionality, and a correction was employed by means of an experimental curve relating deflection to duration of illumination

TABLE III. Gastrocnemius at 9·4° C. in O_2 Ringer: regular series at 1 min. intervals.

Sequence of observations		No. of extra shocks 0·5 sec. apart	Mean supernormal effect of extra shocks p.c.	Average p.c.
1			35	
2			35	34
	12	1	33	
3		5	65	76
	11		86	
4		10	104	117
	10		129	
5		20	136	136
	9		135	
6		30	156	148
	8		140	
7		40	133	133

of the photoelectric cell by an unsymmetrical light spot. See Fig. 3 and Table III.

At higher temperatures the phenomena of the supernormal phase are not so evident, and the time intervals at which they must be sought are much less. At 20° C., for example, an interval of 5 sec. should correspond approximately to one of 1 min. at 0° C. In the paper by Hill [1931 *a*, Fig. 9, p. 296] there are obvious supernormal responses to the later shocks of a 0·9 sec. and a 0·6 sec. series at 20·2° C. Since the

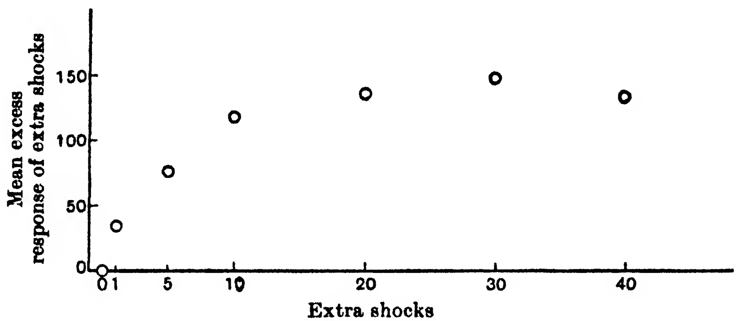

Fig. 3. Constructed from Table III. Horizontally, number of extra shocks at 0·5 sec. intervals given immediately after one of a regular series of shocks at 1 min. intervals; vertically, mean supernormal effect, *i.e.* mean response of extra shocks in excess of response in regular series expressed as a percentage of the latter. Gastrocnemius at 9·4° C. in O_2 Ringer.

intervals required are longer and the phenomena are more evident at the lower temperature, attention in the present research has been directed chiefly to these.

Discussion.

It has been shown that the tension-time response is greater, after a short interval during which it is less, the less complete the return of the muscle is to its resting or steady condition at the moment of response. There are two factors in this increase of the area of the tension-time curve, namely the usual staircase effect and the "slowing" effect recently referred to by Hill [1931 *a*, Fig. 9]. The latter factor seems to be much the more important.

The supernormal phase in the thermal response and that in the mechanical response are independent of each other [see Hartree and Hill, 1921, Fig. 6]. There are various other forms of response in which a supernormal phase has been reported, *e.g.* in the electric response

[Samojloff, 1908; Adrian and Lucas, 1912], in the height of isotonic contraction under certain conditions [Bremer and Homès, 1932] and in the return of excitability [Adrian and Lucas, 1912; Cooper, 1924]. Whether there is any connection between the various supernormal phenomena is not certain.

SUMMARY.

If a regular series of single shocks be given to a gastrocnemius muscle through its nerve its tension-time response increases until a stationary level is attained. Increasing the stimulation interval causes a fall, decreasing the interval a rise in the level, so far as this is not affected by the onset of fatigue or by the relatively refractory period.

The so-called supernormal response, at least in the case of the tension-time of a contraction, can be regarded as the first step towards a change in the steady level due to a sudden change of the stimulation interval.

I am deeply indebted to Prof. A. V. Hill who suggested the method, and under whose kind direction and help the work was carried out. To Mr J. L. Parkinson I wish to express my thanks for his assistance in the technique.

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THE EFFECT OF INJECTIONS OF EXTRACTS OF ADRENAL CORTEX ON THE DEVELOPMENT AND SEX FUNCTIONS OF THE ALBINO MOUSE.

By R. A. CLEGHORN.

(*From the Department of Physiology, The University, Aberdeen.*)

In a previous paper [Cleghorn, 1932] proof is given of the activity of extracts of beef adrenal cortex prepared according to the method of Swingle and Pfiffner [1931]. It was indicated there that observation of the effect of this material on the sex functions supplied an excellent means for the experimental test of the averred relationship between them and the adrenal cortex. This has already been tried but with conflicting results. Connor [1931] found that rats injected with Swingle and Pfiffner's extract matured no faster than controls, as judged by the time of opening of the vagina, vaginal smears in the female and the behaviour of males to females in œstrus. Nor did he find the œstrous cycle of adult rats to be interrupted by injections of the extract. Previously [1930] he had reported that the injection of hens with a crude watery extract of beef adrenal cortex caused cessation of laying, atrophy and degeneration of the ovaries and delay in the development of young chickens. This extract, he claims in his later publication, interrupts the œstrous cycle of rats.

Contrary to Connor's [1931] report are the findings of Corey and Britton [1931 *a*, *b*]. These workers claim to have produced precocious maturation of the sex glands of young albino rats by injections of Swingle and Pfiffner's extract. Changes were not so marked in the testes as in the ovaries. The weight increases of the test animals were from 10 to 18 p.c. greater than those of the controls.

The work herein described constitutes a further attempt at the experimental elucidation of the averred relationship between the adrenal cortex and the gonads, using extracts of adrenal cortex.

MATERIAL.

The albino mouse was selected as the test object on account of its convenient size and easily observed sex phenomena. The chief extract used for injecting the mice was prepared according to the method of Swingle and Pfiffner, to which reference has already been made. A crude watery extract was used in a few experiments and these are detailed in part (*b*).

The mice forming the initial stock were secured from a reputable London dealer. They were kept in a warm room, temperature 70–80° F., and fed regularly on a diet of bread soaked in milk, crushed oats and millet seed, with a constant supply of water. Under these circumstances regular œstrous cycles were observed and no difficulty experienced in breeding the animals. Our experience accords with that of Wyman [1928] and others, namely that low temperature and inadequate diet interfere with the reproductive functions of the rodent.

(*a*) THE EFFECT OF INJECTIONS OF EXTRACTS OF ADRENAL CORTEX ON THE GROWTH AND MATURATION OF MICE.

The criteria selected for judging the effect of the injected extracts were:

(1) Growth (body weight and testis weight).

(2) Maturation. Age of opening of the vagina. This is held to be an accurate index of physiological maturity since, according to Engle and Rosasco [1927], œstrus, and hence ovulation, usually occurs within 24 hours after the vagina opens.

Of the litters of ten mice, totalling 55 young, 39 were used—18 as test, and 21 as control animals.

Two litters born on the same day constituted an experimental unit of test and control groups. There were four such units. The size of the litters was equalized at birth by discarding from the larger, and in three of the units half the young were transferred from the prospective test group to the control group and *vice versa*, in order to control genetic factors which might influence growth and sex development. The litters in the fourth unit were not interchanged, but the results are so similar to the others in detail as to make it probable that consideration of the genetic factor is unnecessary. Two additional litters were observed as extra controls.

All injections were administered subcutaneously and no untoward

reaction was seen to occur. Of the six mice that died two were killed by the needle and four from unknown causes. Injections of beef cortical extract 1/20 c.c. twice daily, *i.e.* the equivalent of the cortex of one beef adrenal (6 g.), were administered throughout the lactation period of 3 weeks to the mother mouse of the test litter. The mother of the control group was injected with extract inactivated by boiling, or with saline in equal amounts. The young mice were weighed on weaning and twice weekly thereafter. Injections were begun on the 21st day, the test mice receiving 1/20 c.c. active extract twice daily for 2 weeks, then 1/10 c.c. twice daily for 2 weeks. The controls were injected with inactivated extract or saline in similar amounts.

Results.

Only the initial and final body weights need be given along with the combined weight of the testes taken at the end of the experiment.

TABLE I.

Exp. No.	Males: Initial wt. (g.) Test	Control	Males: Final wt. (g.) Test	Control	Males: Combined wt. Testes (g.) Test	Control	Females: Initial wt. (g.) Test	Control	Females: Final wt. (g.) Test	Control
I	—	6·0	—	13·7	—	0·12	7·7	7·4	18·4	16·3
	—	7·3	—	19·0	—	0·15	7·8	—	17·6	—
	—	5·8	—	15·4	—	0·12	7·4	—	15·7	—
							7·1	—	14·5	—
							7·2	—	16·2	—
							7·0	—	15·8	—
II	8·1	7·2	17·3	19·0	0·16	0·185	8·0	8·8	20·1	16·0
	7·4	7·9	17·0	16·8	0·15	0·185	7·3	9·3	17·7	16·0
	7·0	—	15·9	—	0·13					
III	4·6	3·8	13·6	12·9	0·10	0·10	5·5	5·5	11·1	13·9
							5·6	3·7	13·8	12·2
							5·5	4·9	Died	13·4
IV	8·2	9·2	14·5	18·2	0·125	0·185	8·0	7·7	16·0	14·4
	8·0	7·7	16·7	18·3	0·125	0·165	—	8·2	—	15·3
Extra		7·1	—	14·6	—	0·12				
Control		6·6	—	13·4	—	0·12				
Group I		7·7	—	12·9	—	0·12				
Average	7·2	6·9	15·8	15·8	0·131	0·143	7·0	6·9	16·1	14·7

Initial weights on 21st day. Final weights in Exp. I on 51st day, others on 40th.
Extra Control Group II is not included here since final weight was taken on 40th day.

Consideration of Table I shows the average weight of the testes of the test animals to be 8·3 p.c. less than that of the controls, and the final weight of the test females 8·7 p.c. greater than the controls.

Table II shows that there was no substantial difference between the test and control groups in either average weight or age at the time of opening of the vagina. The average of 31·5 days for the occurrence of

TABLE II.

Exp. No.	Weights at vaginal opening (g.)		Age at opening (days)	
	Test	Control	Test	Control
I	12·5	13·4	31	31
	12·8	—	31	—
	11·8	—	31	—
	11·8	—	38	—
	11·7	—	38	—
	10·5	—	31	—
II	12·3	10·8	29	30
	12·2	12·4	29	32
III	9·8	10·9	35	37
	9·8	7·5	35	33
	9·6	8·6	33	33
IV	12·7	10·4	29	29
	—	12·6	—	32
Extra Control	—	13·5	—	30
Group II	—	14·5	—	28
Average	11·5	11·4	32·5	31·5

this phenomenon in the controls appears early compared to the range of 6–7 weeks given by Parkes [1929], but is in close agreement with that of Engle and Rosasco [1927], who found that the first appearance of œstrus in 100 mice occurred from the 28th to the 49th day, with a median at the 35th day. This discrepancy may be explained by genetic factors [Mirskaia and Crew, 1930]. It may be mentioned here that the term "sexual maturity" does not necessarily imply the occurrence of first œstrus and ovulation. Parkes [1925] has used it "to mean the capacity of the sex organs to function."

(*b*) THE EFFECT OF INJECTIONS OF EXTRACTS OF ADRENAL CORTEX ON THE ŒSTROUS CYCLE.

The œstrous cycle was observed by the vaginal smear technique in a number of adult female mice for a period of weeks. The periods of cornification, or œstrus, and the diœstrous interval varied in different animals, but the usual length of the cycle was 5–7 days. All injections were made subcutaneously.

(1) *Effect of Swingle and Pfiffner's extract.*

Exp. i. Six mice with regular cycles were injected daily for a week with 1/10 c.c. of extract inactivated by boiling, and then with a similar amount of active extract for 2 weeks. In the chart below the œstrous cycles are recorded just before and during the experiment:

```
Mouse
 No.
  5  00000--0I00--000 | A0--00----------0 | 00------00--0000
  6  0000-I--0000-- | A--------00--0000 | ---0000
 30  0000--00I0------- | A0------000------ | -----000
  1  0000---I-000--- | A000----------0000 | --00
 11  00-0I00---00 | A0---00------000- | -------000
 17  000000--00I0--000- | A-0000--00000-- | 0000
```

0 means œstrus; – diœstrus; I injections of inactivated extract begun; at A daily injection of active extract begun. The vertical line indicates cessation of injections.

The obvious objection to this experiment is that an interval was not allowed between the control and test injections, so that it is difficult to say whether the subsequent disturbances which sometimes occurred in the cycles were due to the specific influence of the active extract or merely to the injections *per se*. The latter interpretation appears probable from the occurrence of the prolongation of the diœstrous interval in No. 30 during the injections of inactivated extract and the absence of any disturbance of the cycle of No. 17 with either type of injection.

Exp. ii. This was performed with five mice, two of which had been used in Exp. i whose cycles were of proved regularity. The remaining three mice had somewhat less regular cycles. They all got 1/5 c.c. of active extract twice daily.

```
Mouse
 No.
  1   00--000A---000------0000 | -----------
  2   0000--A000000---0000 | 00---00000
 32   00--0000-A-00000-- | 000
 33   0---------00A000000-- | --00
 34   0-------0A00---00- | --------- (scabs)
```

The large dose of extract injected did not summarily terminate œstrus in any of the mice, although prolongation of diœstrus is noticeable in No. 1. This mouse looked dirty and unkempt and had but two periods of œstrus in the subsequent 6 weeks. No. 34 developed scabs, sufficient explanation for the prolonged diœstrus, and had to be killed.

It is apparent then that injections of this type of extract of beef adrenal cortex do not immediately terminate œstrus. The frequent prolongation of the diœstrous interval seen during or following such in-

jections cannot be held to be specifically the effect of the extract *per se*, since control experiments in which inactivated extract was used gave similar prolongation, and we have also observed this with saline injections. Connor [1931], too, has recorded a similar occurrence in rats with adrenaline injections.

Connor also reports an experiment in which 13 female rats were injected daily with 2 c.c. of extract made by macerating fresh beef cortex with an equal amount of Ringer's solution and filtering through a Seitz, with the result that œstrus was suppressed in seven, and appeared irregularly in the other five. These results were the reason for the following experiment:

(2) *The effect of watery extract of beef adrenal cortex.*

Exp. For each day's injection of extract fresh glands were secured, except on two occasions when glands were not available, so that extract made on one day had to be used on the second also. Dissected cortex was minced and mixed with an equal amount of Ringer's solution and left in the refrigerator for an hour. Mice Nos. 12, 17, 32, 33 and 35 were injected twice daily for 3 days with 0·3 c.c. of the fluid filtered from the above gland mixture through a coarse filter paper in a Büchner funnel. Subsequently they, and the other mice from the beginning, received 0·4 c.c. twice daily of extract obtained by centrifuging the fluid strained through muslin from the gland-Ringer mixture and filtering through a Seitz.

Since an adult mouse has a weight of about one-seventh that of an adult rat, the amount of the dose is relatively greater than that used by Connor.

The œstrous cycles of the mice before and after injections are recorded below:

<table>
<tr><th>Mouse No.</th><th></th></tr>
<tr><td>12</td><td>000 – 0T0000 – 00 – – dead</td></tr>
<tr><td>17</td><td>0 – 00T000 – 00000 – – – – 0 | 0 – – –</td></tr>
<tr><td>32</td><td>0 – – 0T00 – – – – 0000 | 00000</td></tr>
<tr><td>33</td><td>0 – – – – T000 – – – – – | 00000</td></tr>
<tr><td>35</td><td>0 – T000 – – 00 – – 0 | – – – –</td></tr>
<tr><td>42</td><td>– 0000 – – T00000 | – – – –</td></tr>
<tr><td>52</td><td>00 – – – T0000 – – – – | – – – –</td></tr>
</table>

T means injections started. Vertical line, stopped.

It is evident that in no case did œstrus cease immediately injections were begun, as noted by Connor in his similar experiment with rats. Œstrus also recurred in five of the mice during the period of injections.

In two cases œstrus did not recur during the period of injections or in the days of observation subsequently. There is a tendency to prolongation of the diœstrous interval.

Discussion.

These results indicate that injections of Swingle and Pfiffner's extract of adrenal cortex do not affect growth and maturation in mice, and they are substantially in agreement with those of Connor in which rats were used. The occurrence of considerable individual variation makes questionable the significance of the observations that the average weight of the testes in the test group was 8·3 p.c. less than in the controls, and that there was a preponderance of 8·7 p.c. in body weight of the test females. On the other hand the excess weight of 10–18 p.c., shown by the test groups to controls in Corey and Britton's experiments, may be significant, for these workers also showed that the ovaries of injected animals were stimulated to mature more rapidly than normal. However, the fact that opening of the vagina in our test animals did not occur at an earlier date than in the controls is held to be sound evidence that maturation cannot have been accelerated.

In seeking an explanation for the apparent discrepancy in these results, the question of dose first arises. Corey and Britton record early maturation and 10 p.c. weight excess in litters 11 and 13 in which the dose was 1 c.c. on alternate days. The average weight of these rats was about 50 g. The average weight of the mice at the same age (5 weeks) was about 13 g., the dose 1/10 c.c. daily, though they subsequently received twice this amount. These are closely comparable doses, presuming that the extracts were of equal strength, and as shown elsewhere [Cleghorn, 1932] the extract used in these experiments was effective in combating adrenal insufficiency in cats; as little as 7·5 c.c. restoring a prostrate cat to activity. At the same time it must be noted that the extracts used in the foregoing experiments contained a substance, probably histamine, having a deleterious effect on young rats and mice when injected intraperitoneally, and that this may have masked any growth or maturation effect that might otherwise have been evident.

Concerning the effect of cortical extracts on the œstrous cycle, the results with the Swingle and Pfiffner extract reported here are in accord with Connor's findings. Such disturbances of the œstrous cycle as are seen in some of the mice may be fairly considered non-specific for reasons stated. The marked interference with the cycle of rats

injected with watery extract reported by Connor is not confirmed by similar experiments with mice. We venture to suggest that the interference may be due to non-specific causes and not evidence of a "sex-affecting portion" of the adrenal cortex.

Summary.

1. The final average weight of young female mice, injected daily for a period of 4 weeks with an extract of adrenal cortex made according to the method of Swingle and Pfiffner, is 8·7 p.c. greater than the controls. There was no observable difference in the final average weight of test and control males. The final average weight of the testes of the test males is 8·3 p.c. less than that of the controls. These weight differences are not held to be significant.

2. The injection of this type of extract has no appreciable influence on the age of maturation of young female mice as judged by the opening of the vagina, nor has it any specific effect on the sex function of the adult female mouse as judged by its effect on the œstrous cycle.

3. The injection of a watery extract of beef adrenal cortex does not cause the cessation of œstrus, and the frequent prolongation of the diœstrous interval that follows such injection can only be attributed to non-specific influences.

I wish to thank Prof. J. J. R. Macleod, F.R.S., for his helpful criticism, and Dr A. S. Parkes for advice on several points.

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THE REFRACTORY PERIOD OF MAMMALIAN CARDIAC MUSCLE, WITH ESPECIAL REFERENCE TO PURKINJE TISSUE.

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The observations of Mines [1913] on the frog's heart suggest that the refractory period of the ventricular muscle shortens as the rate of beating is raised. Lewis, Drury and Bulger [1921] demonstrated this relation in the auricular muscle of the dog, while recently Tso [1930] has published some observations on the frog's ventricle which confirm the findings of Mines [1913].

That the refractory period decreases as the rate of beating is raised, appears to be a property of cardiac muscle.

In all these observations the stimulating shocks, which test the refractory period, have been sent in at one point, and the response of the muscle has been recorded either from paired electrodes placed a short distance away on the muscle [Lewis, Drury and Bulger, 1921] or from electrodes placed upon the base and apex of the heart [Mines, 1913; Tso, 1930]. A conduction factor is therefore introduced, and the refractory period measurement is not necessarily of the earliest moment at which the muscle beneath the stimulating electrodes will respond, but of that at which the response will travel to a distance through the muscle. From the point of view of the heart beat, this measurement is of interest, but the conduction factor must not be overlooked when the actual properties of the muscle cell are under consideration. It is probable that, under physiological conditions, identical measurements will be obtained with the above method and with those in which the response is recorded at the actual point of stimulation [Andrus and Carter, 1930]. When the heart is poisoned by drugs the two measurements will differ, for it has been shown by Drury and Love [1926] that, in the veratrine poisoned frog's heart, the muscle beneath the

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stimulators responds at a moment considerably earlier than that at which its response can travel even a short distance away.

To distinguish between these two refractory period measurements Lewis and Drury [1926] suggested the terms "effective refractory period" for those measurements in which the response must be conducted to a distance, reserving the term "absolute refractory period" for measurements in which the conduction factor is eliminated.

In this paper we are primarily concerned with observations upon the refractory period[1] of the perfused rabbit's ventricle. In earlier experiments we were unable to find the accepted relationship between refractory period and the rate of beating. We thought that this might be due to the fact that the nutritive and mechanical conditions are very different in the perfused organ, but this conclusion was not supported by further experiments. These and associated experiments have led us to conclude that, in such a preparation, the refractory period measurements obtained are not those of ventricular muscle but of a mixed tissue containing muscle and Purkinje fibres.

THE PERFUSED RABBIT'S VENTRICLE.

The rabbits were killed by a blow on the head, and the hearts quickly removed from the thorax. They were perfused in the usual manner through a cannula inserted into the aorta, with a Ringer's fluid of the following composition: NaCl, 0·92 p.c.; KCl, 0·04 p.c.; $CaCl_2$, 0·024 p.c.; $NaHCO_3$, 0·015 p.c. This fluid was brought to a pH of 7·2–7·4, and was continually oxygenated by bubbling oxygen through it.

Although such evidence as exists strongly suggests that the vagus fibres do not influence the ventricular muscle [Drury, 1923; Rothberger and Scherf, 1930; Dale, 1930], it has been shown that the rhythmic shocks stimulate the vagal fibres in the auricular muscle of the dog and definitely influence the refractory period [Lewis, Drury and Bulger, 1921]. Atropine sulphate was added as a measure of precaution, in doses of 0·2–1 c.c.of a 1 p.c. solution to each litre of perfusion fluid.

The refractory period was measured in the following manner. Fish-hook electrodes were fixed into the epicardium of either the right or left ventricular muscle and paired non-polarizable electrodes connected to a galvanometer string applied a short distance away. The heart was

[1] All our measurements are of the "effective refractory period" except those in which the contrary is stated.

driven rhythmically from the fish-hook electrodes and an extra shock about ten times threshold value passed through the same electrodes, using the apparatus already described [Drury, 1925]. With a double string carrier in the galvanometer the shocks were recorded with one string, and the response of the ventricle simultaneously with the other[1]. To maintain a zero pressure in the ventricles, openings were freely made into the chambers. The sino-auricular node was cooled by placing a small metal tube upon it, through which ice-cold water flowed, or alternatively the A.-V. bundle was cut. This gave a slow natural rhythm and allowed the period to be tested at low rates of beating. As the preparation is slowly deteriorating, and the temperature of the heart susceptible to change with varying coronary flow, observations over a range of rates must be made as quickly as possible. It is only justifiable to compare values obtained in such a series and not those obtained at different stages of the same experiment.

The results typical of such experiments are shown in Table I. From these measurements it is clear that the absolute refractory period of both right and left ventricular muscle remains relatively constant over a considerable range of rates. In some experiments (0.8.12 *a* and *b*), a slight decrease in the period occurs as the rate of beating is raised, but it is not so great as would be expected for the heart *in situ* over the same range of rates.

The perfused heart, even with a well-oxygenated saline, must be considered relatively anoxæmic. To determine whether this was a factor in the results, blood Ringer was used as a perfusate. The animal was anæsthetized and bled from the carotid artery prior to removal of the heart. This blood was defibrinated and added to the perfusate, in amounts varying from 20 to 30 c.c. to 1 litre of Ringer's solution. The measurements obtained in these experiments did not differ materially from those obtained from hearts perfused with Ringer's solution. In two experiments the heart was perfused with whole defibrinated blood, for apart from the question of oxygen supply the saline perfusates differ from blood not only in their chemical composition but also in their viscosity, and it was thought that this might exert an influence. It was found possible, by returning the blood to the perfusion bottle immediately it left the heart, to work with 200 c.c. of defibrinated blood. Of this 150 c.c.

[1] In later experiments, the second string was dispensed with, the primary current of the stimulators being passed through a low-resistance loud speaker unit. The movements of the reed corresponding to the passage of the current were photographed simultaneously with the record of the galvanometer string.

TABLE I.

Exp. 0.8.1. Right ventricle.

Rate per min.	72	136	67	94	120	200
Response	0·135	0·133	0·139	0·134	0·136	0·137
No response	0·103	0·122	0·109	0·125	0·126	0·118
R.P.* in sec.	0·119	0·127	0·124	0·129	0·131	0·126

Exp. 0.8.5. Right ventricle.

Rate per min.	79	84	84	167	214
Response	0·21	0·22	0·20	0·21	0·21
No response	0·16	0·18	0·19	0·20	0·19
R.P. in sec.	0·185	0·200	0·195	0·205	0·200

Exp. 0.8.12. (*a*) right ventricle; (*b*) left ventricle. Atropine added.

	(*a*)		(*b*)	
Rate per min.	107	214	86	197
Response	0·226	0·196	0·250	0·212
No response	0·211	0·185	0·230	0·182
R.P. in sec.	0·218	0·190	0·240	0·197

Exp. 0.8.12 *a*. (*a*) right ventricle; (*b*) left ventricle. Atropine added.

	(*a*)				(*b*)		
Rate per min.	60	80	115	120	58	77	125
Response	0·192	0·198	0·185	0·188	0·222	0·209	0·192
No response	0·177	0·172	0·172	0·168	0·204	0·185	0·175
R.P. in sec.	0·185	0·185	0·178	0·178	0·213	0·197	0·183

Exp. 0.8.14. (*a*) right ventricle; (*b*) left ventricle. Atropine added.

	(*a*)			(*a*)		(*b*)		(*b*)	
Rate per min.	83	166	250	107	214	100	200	100	200
Response	0·120	0·128	0·115	0·135	0·132	0·178	0·174	0·182	0·174
No response	0·107	0·124	0·104	0·119	0·121	0·176	0·157	0·171	0·160
R.P. in sec.	0·113	0·126	0·109	0·127	0·126	0·177	0·164	0·176	0·167

* This figure is the mean between the longest "no response" and the shortest "response." As each successive testing shock is usually delayed by about 0·01–0·020 sec., the error may amount on occasions to, but not more than, 0·020 sec. In subsequent tables the mean figure is alone tabulated.

were obtained from the ear veins of five rabbits, and another 50 c.c. from the rabbit supplying the heart for perfusion, by bleeding it from the carotid artery, introducing saline intravenously, and rebleeding. The different samples were defibrinated and mixed. The heart was removed, washed through with saline, and then connected with the perfusion apparatus. The perfusion circuit was continuously maintained by catching the blood immediately it had left the heart, and carrying it up a glass tube into the perfusion bottle, by means of a continuous stream of oxygen bubbles. The perfusion pressure obtained by this method was about 40 mm. of mercury, and the hearts were beating well at the end of 2 hours. The measurements of the refractory period which were made at different intervals after perfusion commenced showed that such a

preparation did not differ from those perfused with saline (Table II). It is evident therefore that neither anoxæmia, the lessened viscosity, nor the chemical constitution of the saline perfusates can be considered the cause of the constant refractory period.

TABLE II.

Exp. 1.12.9. Rabbit's heart. Blood perfusion.

Rate per min.	290	310	380	450	300	360	280	178	216	300
R.P. in sec.	0·102	0·105	0·103	0·107	0·101	0·103	0·126	0·132	0·141	0·131

The whole perfused heart presents practical difficulties for two reasons. Firstly, very low rates of beating cannot be tested, and secondly, at high rates ventricular fibrillation is often set up by the testing shocks which fall immediately after the refractory period has terminated.

A few observations therefore have been made upon muscle strips. Such preparations possess the advantages that they are quiescent and that ventricular fibrillation does not develop readily. The strips, prepared in the manner already described [Dale, 1930], were immersed in a saline bath, kept at a temperature of 37° C. They were placed resting on the bottom of the bath in a horizontal position, and two parts of the epicardium—namely that stimulated and that to which the recording electrodes were applied, were kept just above the level of the saline. The results, as is seen from the measurements given in Table III, show no

TABLE III.

Exp. 1.1.23 *b.* Left ventricular strip.

Rate per min.:	27	21	43	75	35	50	100	60	120	75	15	30	150	75
R.P. in sec.:	0·19	0·20	0·19	0·17	0·16	0·17	0·17	0·17	0·17	0·18	0·17	0·18	0·16	0·17

Exp. 1.2.19 *b.* Left ventricular strip.

Rate per min.:	50	150	300	62	187	374
R.P. in sec.:	0·08	0·08	0·09	0·08	0·08	0·08

appreciable difference from those obtained from the whole heart, the refractory period remaining constant over a considerable range of different rates, though there is a tendency for the duration to be slightly less at high rates than at low rates of beating.

THE INTACT RABBIT.

As the above results did not agree with those found in the frog's ventricle and the dog's auricle it seemed desirable to measure the period in the intact rabbit.

The rabbits were anæsthetized with urethane injected subcutaneously, followed, if necessary, by ether. Under artificial respiration, the chest was opened and the pericardium cut. The free edges were sutured to the chest wall making a sling in which the heart rested. The fish-hook stimulators were fixed in the epicardium of the right or left ventricle and the recording electrodes placed a short distance away on the same chamber. The natural heart rate was slowed by cooling the sinus or by burning it with a cautery. As the latter procedure is liable to perforate the auricle, the cooling method is preferable. Atropine sulphate (½ c.c. of a 1 p.c. solution) was injected intravenously. The results in such a preparation usually showed that the length of the period changed as the rate was raised. In certain animals, however, measurements were obtained which indicated that the period was relatively constant, but these results were unusual. In Table IV the measurements typical of these

TABLE IV.

Exp. 1.2.6. Intact rabbit. Atropinized.

Rate per min.:	88	167	176	190	200	200	214	230	250	273	273	375
R.P. in sec.:	0·173	0·122	0·133	0·132	0·111	0·110	0·118	0·107	0·107	0·091	0·096	0·086

Exp. 2.1.19. Intact rabbit. Atropinized.

Rate per min.:	150	200	240	272
R.P. in sec.:	0·133	0·123	0·127	0·123

two results are shown, and it is seen that when the period changes with the rate of beating, the duration is considerably reduced and the reduction is of an entirely different order from that seen in the perfused heart. In Fig. 1 the results from three experiments are plotted, and the curve from the intact animal corresponds very closely to that found for the mammalian auricle [Lewis, Drury and Bulger, 1921]. The curves from the perfused ventricle or ventricular strip have an entirely different form.

It has been stated that in some intact animals the measurements of the refractory period were relatively constant. In animals which exhibited the usual falling curve with rise of rate attempts were made to bring about a constant measurement by altering various factors. The part played by the sympathetic nerves on the refractory period is relatively unknown, for though the work of Andrus and Carter [1930] suggests that adrenaline reduces it, their results cannot be accepted as complete proof, as the injection of adrenaline enhances the rate of

beating which in itself reduces the refractory period. In all our experiments the ventricle had been driven by rhythmic shocks, which probably stimulate sympathetic nerve endings in the muscle. Any vagal influence due to such stimulation can be prevented by the injection of atropine, but the sympathetic effect cannot be completely eliminated by any such simple method. To remove any stimulating effect of the rhythmic shocks, we have therefore made some observations in the atropinized animal in which the rate of the heart beat has been changed by heating and cooling the sinus node, and the refractory period tested in the ventricle by

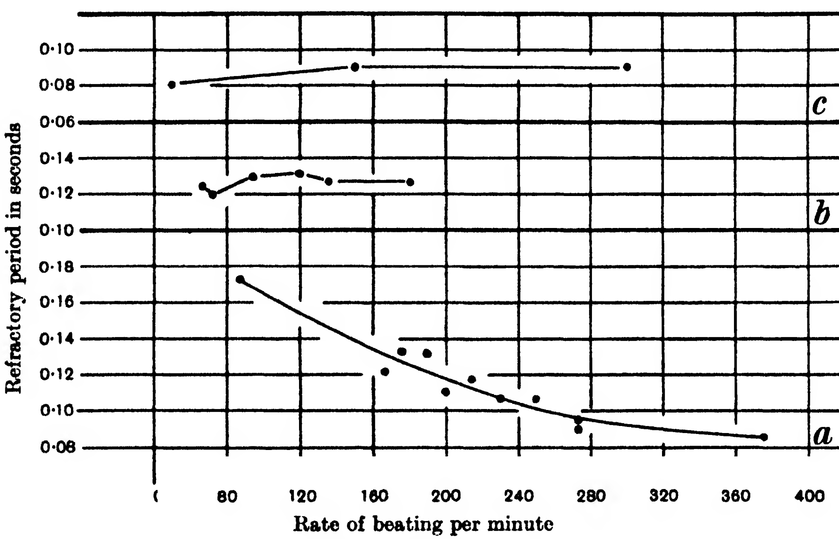

Fig. 1. Refractory period at different rates of beating of the rabbit's ventricle. *a*, intact rabbit; *b*, perfused rabbit's heart; *c*, perfused ventricular strip.

sending in occasional testing shocks through fish-hooks embedded in the epicardium. In others the heart has been driven from one point and the period tested at another distant point on the ventricular surface.

The use of these methods in the mammalian auricle has been criticized [Lewis, Drury and Bulger, 1921]. The criticism is less applicable to the ventricle where the impulse spreads rapidly and practically simultaneously through the Purkinje tissue lining the chambers, and has only to travel through a small thickness of muscle to reach the epicardial surface. In an experiment in which the sinus was cooled and heated, the period measured 0·157, 0·149 and 0·123 sec. at rates of beating of 125, 172 and 225 per min. respectively. When the period was tested at

a point distant from that at which the ventricle was driven rhythmically, it measured 0·138, 0·123 and 0·106 at rhythmic rates of 200, 230 and 300 respectively.

Although ergotoxin has only a slight paralysing effect upon the sympathetic endings in the heart [Otto, 1927] it was thought desirable to determine whether it altered the relation of the period to the rate of beating. The ergotoxin was injected in successive doses of 3·3 mg. till 10 mg. had been introduced. Although the natural heart rate decreased after each injection, the results shown in Table V indicate that this drug

TABLE V.

Exp. 2.1.8. Intact rabbit. Atropinized. Right ventricle.

Rate per min.	206	256	300	333	400
R.P. in sec.	0·121	0·109	0·104	0·094	0·077
3·3 mg. ergotoxin injected*.					
Rate per min.	180	230	250	333	375
R.P. in sec.	0·138	0·117	0·103	0·083	0·094
3·3 mg. ergotoxin injected*.					
Rate per min.	150	200	214	300	375
R.P. in sec.	0·121	0·114	0·100	0·100	0·094
3·3 mg. ergotoxin injected*.					
Rate per min.	150	188	214	250	375
R.P. in sec.	0·140	0·127	0·113	0·112	0·098

* Ergotoxin phosphate (British Drug Houses).

has no effect on the relation between the length of the refractory period and the rate of beating. These two series of experiments are evidence that the relation which is found cannot be due to stimulation of sympathetic nerve endings. There still remained the possibility of a reflex acting through the sympathetic ganglia, and to test this the ganglia were removed in several experiments. Owing to the proximity of small vessels the removal was often accompanied by a slight loss of blood, but on occasions there was no bleeding. Results typical of those given in Table VI were obtained whether hæmorrhage had occurred or not.

TABLE VI.

Exp. 2.1.18. Intact rabbit. Atropinized.

	Normal			Stellate ganglia removed				
Rate per min.	176	214	290	158	171	214	250	274
R.P. in sec.	0·144	0·118	0·106	0·143	0·140	0·137	0·131	0·127

The measurements obtained before and after removal of the ganglia show that the refractory period, after removal, remains practically constant over a considerable range of rates. A slight reduction in the period

at the higher rates of beating is still seen after removal, but this is not of the same order as in the normal animal. It would appear therefore that removal of the stellate ganglia definitely influences the relation between the rate of beating and the length of the refractory period, thus bringing about conditions similar to those found in the perfused organ.

This manœuvre, however, is not the only one which has such an influence. If 50 c.c. of warm saline is injected into the heart through the external jugular vein, the inferior vena cava being obstructed, a similar result is obtained (Table VII).

TABLE VII.

Exp. 2.1.13. Right ventricle. Intact rabbit. Atropinized.

	Normal				After saline injection				
Rate per min.	200	230	260	300	150	182	200	230	150
R.P. in sec.	0·130	0·114	0·109	0·103	0·098	0·115	0·107	0·108	0·117

It would appear therefore that the change in relation between the period and rate of beating may be brought about by both these methods and cannot be specifically associated with the removal of the sympathetic ganglia.

THE CAT'S HEART.

At this stage it seemed desirable to repeat these observations upon another animal, and the cat's heart was investigated.

The cats were anæsthetized with chloralose, and atropine injected. The refractory period was tested upon the left ventricle in exactly the same manner as that described for the rabbit's heart. After the period of the intact heart had been tested, the right and left stellate ganglia were carefully removed, together with their respective thoracic chains as far as the roots of the lungs. The heart rate was slowed by this procedure. The refractory period was again tested. The heart was then removed from the body, and perfused with Ringer's solution at a temperature of 36° C., and a pressure of about 60 cm. of water and the period measured. The values obtained from such an experiment are shown in Table VIII.

TABLE VIII.

Exp. 2.1.21 *b*. Right ventricle. Cat. Atropinized

	Normal			Stellates and chains removed				Perfused					
Rate per min.:	200	250	290	176	200	230	260	158	182	207	230	250	172
R.P. in sec.:	0·120	0·109	0·106	0·160	0·160	0·151	0·138	0·160	0·140	0·123	0·120	0·104	0·138

It is seen that with the normal atropinized heart the period shortens as the rate of beating is raised. After removal of the stellate ganglia, and their associated thoracic chains, the period, though longer, still shortens as the rate of beating is increased, as in the preceding observation. With the perfused heart, the period is shorter, but the relation between the length and the rate of beating is undisturbed.

In the cat therefore no changes are found either after stellate removal or during perfusion, which are comparable with those seen in the rabbit's heart.

One of the main differences between the hearts of the cat and the rabbit is the thickness of the ventricular muscle. The testing shocks used have been considerably above threshold value, and it was felt that the different results obtained in the two animals might be due to the shocks reaching the endocardial surface in the perfused rabbit's heart, which they fail to do in that of the cat. To test this, the period of the perfused hearts of the cat and dog was tested upon the epicardial and endocardial surfaces. In the experiments upon the cat, the animal was anæsthetized with ether, followed by chloralose. An hour was then allowed for the elimination of the ether, after which a cannula was inserted in the carotid artery and a considerable volume of blood withdrawn. The thorax and pericardium were opened and the heart removed, and perfused in the ordinary manner. Incisions were made into the left ventricle along the septal edges from the apex to the base, and the ventricular flap drawn away from the heart by a ligature, thus exposing the endocardial surface.

The dog's ventricular strip was prepared from an animal anæsthetized with morphia and chloralose. After bleeding from the carotid artery, the thorax and pericardium were opened and a cannula inserted into a branch of the anterior descending branch of the left coronary artery. The area of muscle supplied was determined by injecting saline into the cannula and was cut away from the rest of the heart.

In both instances, fish-hook electrodes were embedded in the endocardial surface which was kept as dry as possible by draining away the perfusate by means of cotton-wool wicks. The point chosen depended upon the experimental conditions, both the septum and the endocardial surface of the ventricular wall being used. A pair of fish-hook electrodes was also embedded in the epicardium of the same ventricle, and the recording electrodes placed a short distance away. The period was measured at different rates of rhythmic stimulation, the two surfaces being tested immediately after one another. The results are shown in Table IX, and it is evident that the measurements on the epicardial surface exhibit

TABLE IX.

Exp. 2.1.28. Perfused cat's heart. Left ventricle. Atropinized.					
Rate per min.	176	200	222	250	
R.P. in sec. (epicardium)	0·138	0·131	0·109	0·101	
R.P. in sec. (endocardium)	0·110	0·110	0·096	0·092	
Exp. 2.1.29. Perfused cat's heart. Left ventricle. Atropinized.					
Rate per min.	143	176	214	260	300
R.P. in sec. (epicardium)	0·146	0·137	0·124	0·117	0·099
R.P. in sec. (endocardium)	0·127	0·136	0·134	0·123	0·113
Exp. 2.2.10. Perfused dog's left ventricular strip. Atropinized.					
Rate per min.	100	133	182	240	300
R.P. in sec. (epicardium)	0·180	0·176	0·148	0·116	0·096
R.P. in sec. (endocardium)	0·132	0·108	0·109	0·095	0·092

the usual relation to the rate of beating while those on the endocardial surface remain relatively constant. They also suggest that the period is shorter in the endocardial surface at the low rates of beating. Though this may be actually correct, as we have no evidence as to the temperatures of the two surfaces, our results offer no final proof of the relative durations of the refractory periods.

These experiments lend support to the idea that the constant period in the perfused rabbit's heart is due to the testing shocks reaching the endocardial surface, which they fail to do in the perfused cat's heart. If this explanation is correct, it should be possible to demonstrate the difference between the period of the epicardial and endocardial surfaces both in the intact rabbit and cat. The endocardium has been tested by means of special stimulating electrodes. These consist of fish-hooks which have been straightened out, and everywhere carefully insulated, except on the internal surface of the barbs themselves. Two such electrodes were thrust through the lateral wall of the left ventricle about 5 mm. apart and drawn back till the barb caught the endocardium. When these stimulators are used, the heart is driven from the endocardial surface. Fish-hook stimulators were also embedded in the epicardium, and recording electrodes placed upon the muscle close by. The period was measured at the endocardial and immediately afterwards at the epicardial surface for each rate. In Table X the measurements found in

TABLE X.

Exp. 2.2.1. Intact rabbit. Atropinized. Left ventricle.						
Rate per min.	158	200	272			
R.P. in sec. (epicardium)	0·152	0·140	0·118			
R.P. in sec. (endocardium)	0·153	0·148	0·141			
Exp. 2.2.2. Intact cat. Atropinized. Left ventricle.						
Rate per min.	142	150	167	200	230	273
R.P. in sec. (epicardium)	—	0·208	—	0·172	0·151	—
R.P. in sec. (endocardium)	0·168	—	0·177	0·167	0·163	0·164

the intact rabbit and cat are shown. The observations clearly indicate that while the measurements from the epicardium shorten as the rate is raised, those from the endocardium remain relatively constant. That constant periods are found on the epicardial surface of the perfused rabbit's heart appears to be due to the fact that the testing shocks, on account of the thin ventricular walls, reach the endocardial surface. Judging from the results upon the intact rabbit the use of whole blood as a perfusate might prevent this spread, but in our experiment in which the constant period measurements were still obtained with whole blood perfusion, the heart had first been thoroughly washed through with saline, which is sufficient to render the measurements relatively constant (see Table VII).

The endocardium is lined with a complicated network of Purkinje fibres, and it seems reasonable to conclude that this tissue is responsible for the constant period measurements. To test this point, experiments were performed on the perfused hearts of the dog and the cat in which guarded electrodes were placed beneath the free strands of Purkinje tissue (false tendons) which bridge the endocardial surface of the ventricle. In the experiments on the dog a perfused ventricular strip, as described above, was used. In the cat, the heart was perfused from the aorta, and the ventricular chambers opened. The preparation was driven rhythmically from the electrodes on the Purkinje strand and the testing shocks were sent in through the same pair of electrodes. The responses were recorded from electrodes placed upon the epicardial surface. The measurements from several experiments are given in Table XI (*a*). They show that while over considerable ranges of rate the period is relatively constant, at low rates it lengthens. The lengthening appears to us to be due to the method employed. The response to the first effective stimulus is unable to await the responsiveness of the muscle, the refractory period of which, at low rates of beating, appears to be longer than that of the Purkinje tissue[1]. If the testing shock has produced a localized effect, it may be expected to disturb the conduction of the next rhythmic response, provided this occurs quickly enough, and it is found that testing shocks which fall within the refractory period as measured by the first effective stimulus, cause the next rhythmic response to be delayed, indicating that local responses to these shocks occurred which were not conducted to the recording electrodes. For instance in Exp. 2.3.22 at rate 182, a testing shock of 0·132 delays the

[1] Further evidence that this is so will be given later as a result of the measurements of the conduction interval between the first effective testing shock and its response.

TABLE XI.

(*a*) *Exp.* 2.2.23. Dog. Perfused right ventricular strip. Atropinized.								
Rate per min.	52	109	182	240				
R.P. in sec.	0·174	0·171	0·194	0·171				
Exp. 2.3.1. Dog. Perfused right ventricular strip. Atropinized.								
Rate per min.	46	92	92	120	172	200	260	300
R.P. in sec.	0·188	0·169	0·141	0·168	0·171	0·141	0·145	0·137
Exp. 2.3.16. Cat. Perfused heart. Atropinized.								
Rate per min.	150	172	200	240				
R.P. in sec.	0·144	0·151	0·145	0·160				
Exp. 2.3.22. Cat. Perfused heart. Atropinized.								
Rate per min.	95	128	150	182	214	272		
R.P. in sec.	0·184	0·174	0·174	0·144	0·134	0·134		
Rate per min.	158	182	222	260	280			
R.P. in sec.	0·196	0·167	0·162	0·159	0·168			
Period tested upon muscle.								
Rate per min.	94	122	200	222	260	300		
R.P. in sec.	0·202	0·195	0·171	0·166	0·146	0·125		
(*b*) *Exp.* 2.4.20. Cat. Perfused heart. Atropinized.								
Rate per min.			94	110	150	200	240	
R.P. in sec. (ordinary method)			0·159	0·143	0·131	0·112	0·113	
R.P. in sec. (new method)			0·112	0·118	0·117	0·112	0·113	

next rhythmic response though it gives no recordable response. To confirm this point, an experiment was performed, using a method which detects the responses of the tissue localized around the point of stimulation [Drury and Love, 1926]. This method was used on the perfused cat's heart, and it can be seen from Table XI (*b*) that while the ordinary method gives increased measurements at low rates of beating, constant values are obtained by the new method. It can be concluded therefore that over a considerable range of rates the refractory period of the Purkinje tissue has a constant value.

THE REFRACTORY PERIOD OF VENTRICULAR MUSCLE.

In animals with a thick ventricular wall such as the dog and the cat, the period measurements of the epicardial surface exhibit the same relationship to the rate of beating as that described for the dog's auricle [Lewis, Drury and Bulger, 1921] and the frog's ventricle [Mines, 1913; Tso, 1930]. In addition, we have shown that this relationship which is found in the intact cat is not disturbed when the heart is perfused. The results in such animals can therefore be accepted as measurements of the period of ventricular muscle. Many measurements have been given in this paper for rates above 100 per min. It was thought desirable to obtain measurements at very low rates of beating in addition

TABLE XII.

Exp. 2.2.10. Dog. Perfused left ventricular strip. Atropinized.

Rate per min.	24	60	75	100	115	136	200	272
R.P. in sec.	0·199	0·196	0·197	0·192	0·196	0·165	0·130	0·128

Exp. 2.2.19. Dog. Perfused left ventricular strip. Atropinized.

Rate per min.	22	40	53	68	107	114	130	150	188	250	300
R.P. in sec.	0·172	0·165	0·176	0·166	0·143	0·146	0·137	0·127	0·114	0·112	0·104

Exp. 2.3.1. Dog. Perfused right ventricular strip. Atropinized.

Rate per min.	50	100	133	171	240
R.P. in sec.	0·190	0·195	0·185	0·171	0·151

Exp. 2.3.4. Dog. Perfused right ventricular strip. Atropinized.

Rate per min.	50	100	133	172	222	272	300
R.P. in sec.	0·215	0·210	0·206	0·180	0·152	0·144	0·123

Exp. 2.3.22. Cat. Perfused heart. Left ventricle. Atropinized.

Rate per min.	94	115	162	200	222	260	272	300
R.P. in sec.	0·202	0·207	0·188	0·172	0·166	0·146	0·145	0·136

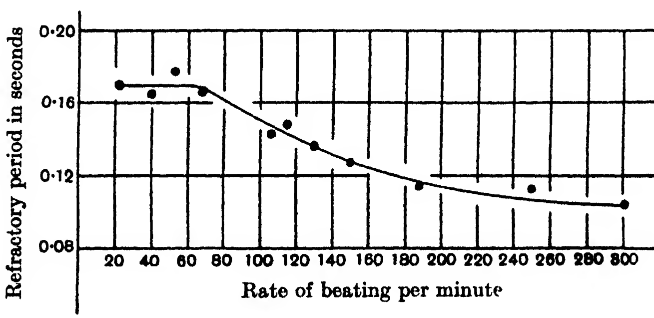

Fig. 2. Refractory period at different rates of beating of the dog's ventricular muscle (perfused strip).

to those at high rates to see whether the refractory period continues to increase, as it does between 200 and 100 per min. Such measurements cannot be obtained in the whole heart, for even if the sinus node is cooled, an A.-V. rhythm sets in which is usually about 100 per min. We have used the perfused left ventricle of the dog and the cat. The refractory period was tested upon the epicardial surface of a quiescent preparation in which very low rates of rhythmic stimulation could be used.

In Table XII the measurements of the period from several different ventricular strips are shown. These indicate that from rates of 20 to about 100 per min. the period remains relatively constant, from 100 to 200 per min. it rapidly decreases, while from 200 to 300 per min. the decrease though still definite is relatively slight. The characteristics of the curve are shown in Fig. 2 which is plotted from Exp. 2.2.19.

The conduction rate of the response to the first effective stimulus at different rates of beating.

In many experiments the recording electrodes and stimulating electrodes have been placed about 5 mm. apart upon the epicardial surface. The interval between the first effective shock and its response is a measure of the time taken for this premature impulse to travel from the

Table XIII. Conduction of response to first effective stimulus at different rates of beating.

(*a*) Travelling through muscle only.

Exp. 2.2.10. Dog. Perfused left ventricular strip (atropinized).

Rate per min.	100	133	182	240	300
Conduction time in sec.	0·041	0·040	0·041	0·043	0·040

Exp. 2.3.18. Intact cat. Left ventricle (atropinized).

Rate per min.	137	162	177	220	222	240
Conduction time in sec.	0·040	0·035	0·036	0·040	0·040	0·040

Exp. 2.3.22. Intact cat. Left ventricle (atropinized).

Rate per min.	158	188	214	250
Conduction time in sec.	0·031	0·030	0·031	0·029

(*b*) Travelling from muscle to muscle, *via* Purkinje tissue.

Exp. 2.3.8. Intact cat. Left ventricle (atropinized).

Rate per min.	176	206	240	272	316
Conduction time in sec.	0·104	0·094	0·113	0·124	0·134

Exp. 2.3.16. Intact cat. Left ventricle (atropinized).

Rate per min.	146	171	200	230
Conduction time in sec.	0·085	0·084	0·114	0·122

Exp. 2.3.22. Intact cat. Left ventricle (atropinized).

Rate per min.	182	225	240	272
Conduction time in sec.	0·079	0·077	0·081	0·097

(*c*) Travelling from Purkinje to muscle tissue.

Exp. 2.2.23. Dog. Perfused left ventricular strip (atropinized). Purkinje strand stimulated.

Rate per min.	46	92	120	172	200	260	300
Conduction time in sec.	0·052	0·057	0·047	0·040	0·040	0·043	0·043

Exp. 2.3.16. Perfused cat's heart (atropinized). Purkinje strand stimulated.

Rate per min.	150	172	200	240
Conduction time in sec.	0·145	0·157	0·124	0·083

Exp. 2.3.22. Perfused cat's heart (atropinized). Purkinje strand stimulated.

Rate per min.	105	128	158	182	212	260	280
Conduction time in sec.	0·118	0·107	0·124	0·114	0·112	0·105	0·104

Exp. 2.2.10. Dog. Perfused left ventricular strip (atropinized). Endocardium stimulated.

Rate per min.	100	133	182	240	300
Conduction time in sec.	0·136	0·128	0·119	0·095	0·092

Exp. 2.1.29. Cat. Intact (atropinized). Endocardium stimulated.

Rate per min.	150	171	214	240	290
Conduction time in sec.	0·129	0·126	0·112	0·100	0·106

stimulating to the recording electrodes[1]. The interval has been measured at different rates of beating for each first effective stimulus, and the measurements between the rates of about 100–250 per min., remain practically constant (Table XIII (*a*) and Fig. 4 *a*). The impulse has two possible modes of reaching the recording contacts, it may spread laterally entirely through muscle or it may travel downwards, through muscle to the Purkinje tissue, and spreading through this, finally reach the recording contacts on the surface by coursing again through muscle. As conduction in the muscle is relatively slow compared with that in Purkinje tissue, the measurement will be of the latter path if the distance between the stimulators and recorders is more than twice the thickness

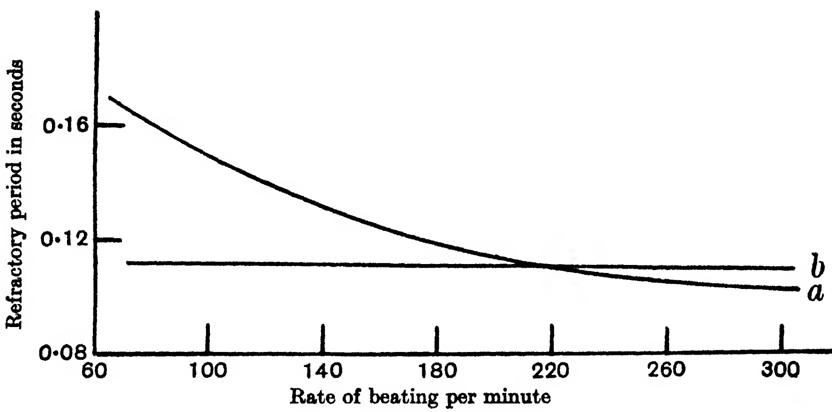

Fig. 3. Diagrammatic representation of the refractory period of (*a*) ventricular muscle, (*b*) Purkinje tissue at different rates of beating.

of the muscle wall. In our experiments, the stimulating and recording electrodes have been placed very close together so that the spread is only through muscle. It can be safely inferred that the rate of conduction through muscle immediately after the refractory period has terminated is constant at different rates of beating.

The measurements of the refractory period of the ventricular muscle and the Purkinje tissue indicate that while the former decrease as the rate of beating is raised, the latter remain relatively constant. In addition they suggest that the values of the two periods at different rates are such that the curves cross between 150 and 250 per min. This relation is diagrammatically illustrated in Fig. 3. Broadly speaking the

[1] This measurement includes the "latency of response." This is, however, in the frog extremely brief [Schellong, 1925].

refractory period of the Purkinje is shorter than that of the muscle at low rates, and longer at high rates of beating. This crossing of the curve should find expression in the conduction intervals of the response to the first effective stimulus at different rates of beating, if it is measured in such a manner that the response has traversed both types of tissue.

When it is travelling from muscle to Purkinje tissue, as the refractory period of the Purkinje tissue is shorter than that of the muscle at low rates of beating, the impulse finds the latter ready to respond. Muscle conduction has been shown to be the same at all rates of beating, so the conduction interval will remain relatively constant. It is true that as progressively lower rates of beating are tested the Purkinje tissue will have a relatively longer period of rest after the refractory period has terminated, and conduction through this tissue will therefore become progressively more rapid. On the other hand, conduction through Purkinje is extremely rapid and represents only a small portion of the total time interval, so that any such shortening might not be appreciated. At rates of beating higher than that at which the two curves cross, the muscle has a shorter period than the Purkinje, and the impulse must use up time travelling in the muscle waiting for the Purkinje tissue to become responsive. The conduction intervals will therefore lengthen, and should become longer as the two periods diverge. This assumes that the first effective impulse in the muscle will always be able to wait for the Purkinje tissue to become responsive, however long that may be. If this condition is not fulfilled, the first effective impulse will die out, and there will be a break in the curve, for the measurements will now apply to the second or even the third effective impulse. In general the measurements of the conduction of the response to the first effective stimulus travelling from muscle to Purkinje should be relatively constant till rates of about 200 per min. are reached when they should lengthen.

Experiments to test this have been made in the following manner. The heart in the intact atropinized cat[1] was driven rhythmically from fish-hook electrodes embedded in the epicardial surface of the left ventricle, while the recording contacts were placed upon the epicardial surface of the right ventricle. This method, however, involves a double passage, namely from muscle to Purkinje and Purkinje to muscle. The impulse liberated from the epicardium of the left ventricle travels from muscle to Purkinje tissue, up the left division of the bundle, over the septum, and down the right division to the muscle underlying the recording contacts on the right ventricle [Lewis and Rothschild, 1915].

[1] In order to obtain low rates of beating the sinus node was either cooled or burnt out.

The second passage, namely from the right division of the bundle to the muscle, will influence the results only at low rates of beating, for the impulse liberated will find the Purkinje tissue in the left ventricle responsive and will here suffer no delay. When it arrives at the junctions in the right ventricle, the muscle will still be irresponsive and a delay will occur. This delay will be greater the lower the rate of beating. At high rates of beating, after the curves have crossed a delay will occur in the left ventricle, as the impulse must await the responsiveness of the Purkinje tissue. As the rate of beating therefore is raised from low rhythms, the measurements will decrease and reach a minimum at the

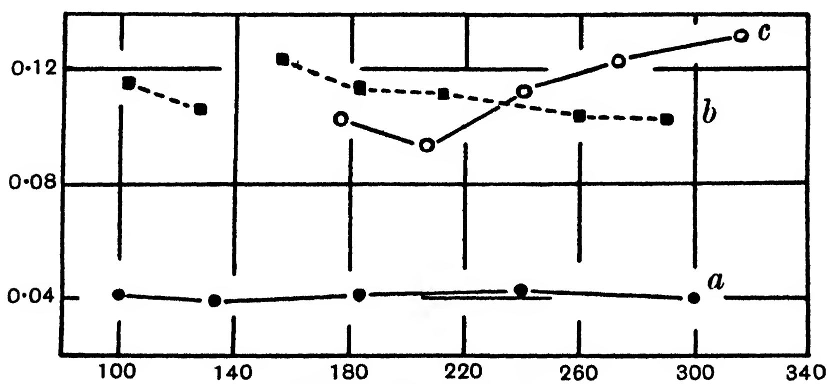

Fig. 4. Conduction of the response to the first effective stimulus. *a*, travelling through muscle; *b*, travelling from Purkinje tissue to muscle; *c*, travelling from muscle to muscle *via* Purkinje tissue.

point where the period curves cross, and then lengthen once more. The results which are given in Table XIII (*b*) and Fig. 4 *c* show that that expected result is realized.

Evidence of the delay of the passage of the impulse from Purkinje tissue to muscle at low rates of beating is provided by the measurements from the following experiments. In these the stimulators were placed upon a Purkinje strand, and the response recorded on the epicardium. In this case there is only one passage, namely from Purkinje tissue to muscle. At low rates of beating, the response in the Purkinje tissue[1] must await the responsiveness of the muscle, and will suffer a delay. After the curves have crossed no such delay will occur, and as pro-

[1] We have no evidence as to the rate at which the response to the first effective impulse liberated in Purkinje fibres travels in this tissue at different rates of beating. We have assumed that it is constant at all rates, as this is found to be the case in muscle.

gressively higher rates are tested the muscle will have a relatively longer period of rest and conduction should be quicker. A further decrease due to this factor might be expected, but at high rates the two curves diverge very slowly owing to the flattening out of the muscle curve, so that this influence cannot be very great. The measurements should therefore decrease definitely as far as the point of crossing of the curve, after which they will remain constant, or if the improvement in muscle conduction comes into play, have slightly lessened values. This result is seen in the measurements from Table XIII (*c*)[1] and Fig. 4 *b*. They show in addition that at low rates of beating an abrupt change may occur in the measurements. This is due to the response to the first effective stimulus in the Purkinje fibres being unable to await the responsiveness of the muscle, so that the measurements are now of the second or third effective stimulus. These observations are evidence that the relation between the refractory period of Purkinje tissue and muscle which was indicated by actual measurement is correct.

The diagrammatic representation of the two periods in Fig. 3 appears to us to represent, reasonably accurately, the relation as it actually exists.

THE INFLUENCE OF DIASTOLIC FILLING UPON THE REFRACTORY PERIOD.

Tso [1930] has shown that variations in the diastolic filling of the frog's ventricle are without influence upon the refractory period provided the rate of beating is maintained constant. These results have also been found by us for the dog's auricular and ventricular muscle. The experiments upon the auricular muscle were carried out on the intact atropinized animal. After exposing the heart in the usual manner, ligatures were placed around the superior and inferior venæ cavæ. These vessels could be closed by tightening these ligatures and the pressure in the right auricle reduced. To produce raised auricular pressure, considerable volumes of saline were injected into the femoral vein. The auricular pressure was measured in some experiments by inserting a wide-bored cannula connected to a saline manometer into the superior vena cava and leading it into the auricular cavity. The diastolic filling could be increased or decreased by these methods, and as the actual measurements of the period were made immediately, it is unlikely that

[1] The measurements at high rates of beating from the experiments in which the endocardium was stimulated are not strictly comparable with those at low rates. The former may represent muscle conduction only as the refractory period of this tissue is shorter than that of Purkinje fibres at these rates.

the results were influenced by the many other changes which must accompany these procedures. The measurements were made upon the right auricular muscle, the stimulating electrodes being placed upon the body of the auricle and the recording electrodes a few millimetres away. The results (Table XIV (*a*)) show that the pressure within the auricle has no influence upon the refractory period at a constant rate of beating, and this holds for considerable variations in venous pressure.

TABLE XIV.

(*a*) *Exps.* 0.10.9, 0.10.14. Intact dog, fully atropinized. Right auricle.

Rate per min.	225	225	225	160	160	160	160
Auricular pressure in cm. of H_2O	22	16	18	13	10	21	13
R.P. in sec.	0·100	0·090	0·086	0·108	0·105	0·103	0·109

(*b*) *Exp.* 1.5.6. Dog's heart-lung preparation. Right ventricle.

Rate per min.:	214	107	214	120	240	240	120	120	120	120	120
Output in c.c. per min.:	250	125	125	150	150	310	310	Small	Medium	Large	Small
R.P. in sec.:	0·14	0·16	0·14	0·17	0·14	0·14	0·15	0·19	0·17	0·17	0·17

The same result can be demonstrated in the dog's heart-lung preparation for the right ventricular muscle. The diastolic filling was changed by altering the venous pressure, and was measured in the experiment by the change in outflow from the left ventricle. The results in such an experiment are tabulated in Table XIV (*b*) and indicate that the period decreases with the rate of beating whatever the venous pressure, and remains constant at the same rate of beating though the venous pressure is changed. These experiments agree with Tso's findings for the frog's ventricle [1930]. It is evident therefore that the refractory period of the auricular and ventricular muscle in the dog is uninfluenced by diastolic filling.

It has been demonstrated in the perfused rabbit's heart that the period measured is not entirely that of ventricular muscle, but also of Purkinje tissue. This tissue is likewise uninfluenced by the initial length to which the muscle is stretched. This was shown in the whole perfused heart by inserting a bladder into the right ventricular cavity connected to a column of liquid paraffin whose height could be increased or decreased. The results (Table XV) show that the period remains constant with the same pressure, though the rate is increased, and also when the rate of beating is held constant and the pressure within the cavity considerably altered. The same result was found for the perfused strip. In this preparation we attached six threads radially which passed over

light pulleys, and on which weights were hung. By altering the weights the muscle could be subjected to different degrees of stretching. The measurements (Table XV) obtained by this method agree with those found for the whole perfused heart.

TABLE XV.

Exp. 1.2.26. Perfused rabbit's heart.

Rate per min.	150	150	150	150	300		
R.P. in sec.	0·152	0·149	0·146	0·184	0·179		
Pressure	Great	Mod.	Slight	Slight	Slight		
Rate per min.	150	150	150	150	150	150	150
R.P. in sec.	0·159	0·165	0·156	0·144	0·140	0·135	0·145
Pressure	Slight	Mod.	Great	Mod.	Slight	Mod.	Great

Exp. 1.2.20. Rabbit's heart. Left ventricular strip.

	Weights attached			No weights		Weights attached		
Rate per min.	43	129	258	43	129	43	129	258
R.P. in sec.	0·14	0·14	0·12	0·17	0·16	0·18	0·15	0·14

	No weights	Small weights	Large weights	No weights	Large weights	Small weights
Rate per min.	136	136	136	136	136	136
R.P. in sec.	0·11	0·12	0·12	0·11	0·12	0·13

The experiments indicate that the refractory period of mammalian heart muscle is uninfluenced by diastolic filling. In addition they show that that of the Purkinje tissue which is responsible for the constant period is likewise uninfluenced.

CONCLUSIONS.

The foregoing observations clearly indicate that the refractory periods of the two tissues, namely muscle and Purkinje, which make up the ventricles have very different characteristics. The period of the Purkinje tissue remains relatively constant, while that of the muscle shortens as the rate of beating is raised. Moreover, there is evidence that the relative durations of the periods is such that at low rates of beating that of the Purkinje fibre is shorter than that of muscle, while at high rates the reverse holds, the curves crossing at rates of 150–250 per min. This crossing of the curve is only seen in occasional experiments on the perfused heart, for it is impossible to maintain the conditions at the Purkinje strand identical with those at the epicardial surface, both as regards temperature and supply of perfusate. In the experiments upon the intact animal, in which both the endocardial and epicardial periods have been compared, the crossing of the curve is not seen for the reason that the testing shocks applied to the endocardium are not limited to Purkinje

tissue only, and they will always measure the shorter period. In such experiments the period measured at high rates is that of muscle, while at low rates it is that of Purkinje tissue. This was indicated in some experiments in the intact cat in which the measurements remained relatively constant till a rate of about 250 per min. was reached, when they shortened abruptly, and followed a curve very similar to that of muscle tissue. In the perfused rabbit's heart, in which we conclude that the testing shocks reach the Purkinje tissue, there has been a tendency for the measurements at high rates to be shorter than those at low rates of beating. This is again due to the fact that at low rates the measurement is that of Purkinje and at high rates that of muscle.

When, in the experiments on the cat and dog, the measurements from the endocardium are compared with those from the Purkinje strands it is seen that in the former case the values remain constant at rates at which a sudden increase is seen in the latter. The reason for the difference between the results is not clear to us, but it may well be due to the fact that nutritive condition of the free strand is not nearly so good as that of the fibres which line the endocardium.

The perfused rabbit's heart and ventricular strips give measurements which remain constant at much lower rates of beating than is the case in the hearts of the dog and the cat when the endocardium is tested. It must be concluded that in the rabbit the impulses liberated in the Purkinje fibres are better able to await the responsiveness of the muscle tissue.

The use of the perfused heart does not alter the characteristics of the period measurements of cardiac muscle, but in the rabbit owing to the thin ventricular wall[1] the shocks are liable to reach the Purkinje fibres, and this gives rise to false results. This may even occur in the intact rabbit. In animals in which the ventricular wall is thick this error does not occur, and the measurements of the muscle period have the same characteristics whether they are tested upon the intact or the perfused heart.

Summary.

1. The refractory period of the perfused rabbit's ventricle, tested on its epicardial surface, remains constant over a considerable range of rates.

2. In the perfused heart of the cat and dog, a constant period measurement is obtained when the endocardial surface or an isolated

[1] The distribution of the Purkinje fibres in the rabbit may differ from that in the cat and dog; they may reach more superficial layers.

Purkinje strand is tested, but the epicardial surface shows the usual relation between period and rate of beating.

3. It is concluded that the refractory period of Purkinje tissue remains constant irrespective of the rate of beating, and that in the perfused rabbit's heart conditions are such that the testing shocks applied to the epicardium reach the Purkinje network.

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THE ACTION OF SOME AMINES RELATED TO ADRENALINE.

I. Methoxy-phenylethylamines.

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The discovery of the composition and structure of adrenaline has led to the investigation of a large number of related compounds in which modifications of adrenaline have been brought about by alteration either of the catechol nucleus or of the methyl-amino-ethanol side chain or of both. Dakin [1905], who was one of the first to explore the actions of compounds related to adrenaline, found that catechol itself produced a rise of blood-pressure in rabbits but that the methyl ether of catechol produced no such effect. He concluded: "It appears that the catechol nucleus is essential for the production of physiologically active substances of the type of adrenaline," and "It is of importance that the hydrogen atoms of both hydroxyl groups in the catechol nucleus be un-substituted."

Later Barger and Dale [1910], in their searching examination of the factors responsible for the adrenaline type of action, showed that the rise of blood-pressure produced by catechol was due to a general stimulation of plain muscle and that catechol itself had not the specific actions of adrenaline.

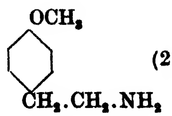
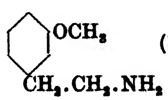
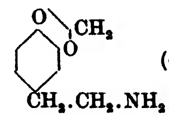
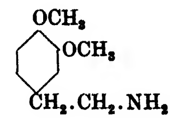
Though the methyl ether of catechol is itself inactive, it does not, therefore, follow that substitution of the hydroxy groups of the catechol nucleus in an adrenaline type of compound will also annul its activity, because catechol does not possess the adrenaline type of action. Nevertheless, it is probable that Dakin's suggestion (that, for the adrenaline type of action, it is important that the hydroxyl groups in catechol nucleus be unsubstituted) has had the effect of deterring subsequent workers from examination of compounds related to adrenaline in which this substitution has been made. Indeed, among the large number of

[1] The compounds were prepared by C. J. Virden and the pharmacological actions investigated by D. Epstein and J. A. Gunn.

substances related to adrenaline which have been investigated, we have found no records of the pharmacological examination of the compounds which are here described.

The primary object of the present investigation was to test the activity of this type of compound, and the following compounds, which were prepared in this laboratory by Dr Virden, have been investigated:

(1) *p*-methoxy-phenylethylamine.
(2) *m*-methoxy-phenylethylamine.
(3) Methylene-dioxy-phenylethylamine.
(4) Dimethoxy-phenylethylamine.

(1)  (2)  (3)  (4) 

The first compound is the methylether of tyramine, the actions of which have been the subject of numerous investigations.

The effect of etherification of a phenolic-hydroxyl group on the physiological activity of a particular compound is difficult to predict. It may lead to a diminution, to an increase, or to a change in activity. In the particular group of compounds under consideration there was reason to expect that such etherification would lead to a diminution of activity, but, as we shall show, it actually led to quantitative and qualitative changes in activity that were hardly to be anticipated. Indeed we found to our surprise that the fourth compound which approximated most closely to adrenaline in structure was the farthest removed from it in physiological action.

In the investigation of the compounds mentioned we had a secondary —and from the therapeutic point of view more important—object in mind, namely, the endeavour to discover a compound which might combine some of the advantages of adrenaline and ephedrine. In the treatment of asthma, for example, adrenaline has the merits of speed and certainty of action; but has the disadvantages that its effects are transient and that it has to be given by injection. Ephedrine produces a more prolonged action and can be given by mouth, but may produce undesirable side-effects, *e.g.* prolonged rise of blood-pressure, with nausea, headache, palpitation and insomnia. The instability and brevity of action of adrenaline is chiefly due to the presence of the two phenolic-hydroxyl groups, and the comparative stability of ephedrine to their absence. The comparative diminution in absolute activity of ephedrine is not a serious dis-

advantage, as it merely means that it has to be given in a larger dose than adrenaline. It was possible that, by adopting some other method of stabilizing the nucleus, one might obtain a substance which would possess some of the attributes of ephedrine without its disadvantages; and methylation of the hydroxyl groups was an obvious method of stabilizing the nucleus and of producing a substance sufficiently resistant to chemical change to suggest that it might produce its actions when given by mouth and produce effects more enduring than those of adrenaline; provided always that this etherification did not stabilize the substance to the point of annulling physiological activity.

TABLE I. Minimum lethal dose for mice, by intraperitoneal injection.

Drug used	Wt. of mouse in g.	Dose per kg. in g.	Actual dose in g.	Result
p-methoxy-phenylethylamine (1)	30	0·3	0·009	Died in 7 minutes
	29	0·24	0·007	Died in 8 minutes
	23	0·22	0·005	Died within 30 hours
	25	0·2	0·005	Died within 2 days
	19	0·17	0·0033	Died within 2 days
	22	0·15	0·0033	Died within 3 days
	27	0·11	0·003	Recovered
m-methoxy-phenylethylamine (2)	22	0·45	0·07	Died in 8 minutes
	26	0·31	0·008	Died in 7 minutes
	35	0·23	0·008	Died in 8 minutes
	25	0·2	0·005	Recovered
	23	0·17	0·004	Recovered
Methylene-dioxy-phenylethylamine (3)	18	0·3	0·0054	Died in 11 minutes
	27	0·26	0·007	Recovered
	23	0·2	0·0046	Recovered
Dimethoxy-phenylethylamine (4)	27	0·55	0·015	Died in 18 minutes
	29	0·5	0·015	Died in 9 minutes
	38	0·42	0·016	Died in 17 minutes
	27	0·4	0·011	Recovered
	34	0·36	0·012	Recovered
	32	0·31	0·010	Recovered
	26	0·19	0·005	Recovered
p-hydroxy-phenylethylamine (tyramine) (5)	21	0·8	0·017	Died in 3 days
	23	0·65	0·015	Recovered
	26	0·5	0·013	Recovered
	24	0·33	0·008	Recovered
	22	0·23	0·005	Recovered
	25	0·16	0·004	Recovered

The present investigation seems to have gone some way towards finding such a substance. It has also revealed some unexpected difference not only between these compounds and adrenaline but between the compounds themselves. These differences concern especially actions on the central nervous system and the so-called "sympathomimetic" actions.

It will be convenient first to compare the toxicities of the compounds

and to describe briefly the general effects produced by them in the intact animal: and subsequently to discuss the actions on various organs with special reference to sympathomimetic action.

In order to obtain some idea of the relative toxicity of these compounds and of the general effects produced by them, experiments were made on mice, the amines being given by intraperitoneal injection. By this type of experiment the maximum amount of information in regard to laboratory mammals can be obtained with a limited supply of drug.

From Table I it can be seen that the approximate minimum lethal dose per kg. for mice by intraperitoneal injection is as follows:

	g.	Ratio
(1) *p*-methoxy-phenylethylamine	0·15	1·0
(2) *m*-methoxy-phenylethylamine	0·23	1·5
(3) Methylene-dioxy-phenylethylamine	0·30	2·0
(4) Dimethoxy-phenylethylamine	0·42	2·8
(5) *p*-hydroxy-phenylethylamine	0·8	5·3

Several points of importance emerge from these results, which incidentally emphasize the importance, in any comparison of a homologous series of drugs, of experiments on the intact and unanæsthetized animal. Often, when such a series of drugs is being compared, this comparison is based upon a single criterion of physiological activity (*e.g.* effect on blood-pressure), and it is at least implied that the data afforded by this single criterion give satisfactory *prima facie* evidence for their relative general activities. The fallacy of such an assumption can be illustrated by a comparison of *p*-methoxy-phenylethylamine (1) with *p*-hydroxy-phenylethylamine (5). When injected intravenously the latter has a more powerful action on the blood-pressure than the former and might therefore be said to be more active, but, when given by intraperitoneal injection in the intact animal, the former is at least four times as toxic as the latter.

Another point of interest relates to the time of death. In the case of compounds (2), (3) and (4), if the animal dies, death occurs within a few minutes, but with compounds (1) and (5) there is a more chronic form of poisoning in which the animal dies after two or three days. It may be of some significance that both these compounds (1) and (5) are para compounds.

As will be shown later, compounds (1) and (2) seem to be, quantitatively and qualitatively, indistinguishable as regards their effects on blood-pressure and on isolated organs. If, in the case of intraperitoneal injections in the intact animal, only those cases be considered in which

death occurs in a few minutes, the toxicities of both are, within experimental limits, also identical (24 : 23). On the other hand, if the animals were kept under observation for several days, it was found that the para compound is considerably more toxic than the meta compound by reason of the delayed poisoning that it produces. It is a remarkable fact that, of the two isomeric compounds which differ chemically only in the position of the methoxy group and which resemble one another otherwise so closely in their physiological action, one should produce a type of delayed poisoning from which the other seems to be entirely free.

Symptoms produced.

(1) p-*methoxy-phenylethylamine.*

The chief symptoms produced were irregular and jerky contractions of the voluntary muscles, especially of the head, with ataxia and loss of power of progression. The animals showed a characteristic type of tremor which resembled coarse shivering. When death occurred within a few minutes, it was due chiefly to respiratory failure, and was preceded by cyanosis and weak asphyxial convulsions. The heart continued to beat feebly for some minutes after respiration ceased. If the dose was just sufficient to produce delayed death, the tremors and ataxia subsided in an hour or so, after which the animal might remain apparently normal for 24 hours or more. Then a type of paralysis came on, characterized by a great fall of temperature and by extremely slow contractions of the voluntary muscles.

(2) m-*methoxy-phenylethylamine.*

With the meta compound the delayed type of poisoning did not occur, and it differed otherwise in its acute effects on the central nervous system. The chief differences were that the meta compound produced more marked acceleration of respiration and increased excitability of the spinal reflexes. While, therefore, the para compounds seemed to excite the motor cortex of the brain, the meta compound, in addition, stimulated the respiratory centre and spinal cord.

(3) *Methylene-dioxy-phenylethylamine.*

This compound produced symptoms, intermediate between those produced by compounds (1) and (2). Certain movements which were characteristic of (2) were reproduced, but there was less increase of spinal excitability than was seen with (2).

(4) *Dimethoxy-phenylethylamine.*

This compound produced quite different effects from the previous three. There were no marked signs of increase of excitability of the motor cortex of the brain or of the spinal cord. The animals became gradually paralysed and died from respiratory failure.

A full comparison of the general effects of the four compounds would require detailed and lengthy protocols. It may suffice to point out that, in this group of compounds which possess an identical ethylamine side-chain, relatively small differences in the structure of the nucleus produce considerable changes in the action of the resulting compounds on the central nervous system. Compounds (1), (2) and (3) resemble one another fairly closely, but (4) is quite different. It will be shown later that, in its other actions also, (4) produces quite different effects from (1), (2) and (3).

Action on the isolated mammalian heart.

Compounds (1), (2) *and* (3).

The hearts were perfused by means of Gunn's apparatus. It was found that compounds (1), (2) and (3) produced a typical sympathomimetic effect (augmentation and acceleration) on the cat's heart but no such effect on the heart of the rabbit. Fig. 1 shows the effect of the same concentration of *p*-methoxy-phenylethylamine (1 in 20,000) on the hearts of the cat and rabbit. The characteristic sympathomimetic effect is produced in the former, but no effect at all on the latter. The same difference in response was seen with compounds (2) and (3).

This difference in response was not merely a quantitative one, because, however high the concentrations used, the sympathomimetic action was absent in the rabbit's heart. This is unmistakably shown in Fig. 2. The upper tracing shows that even 1 in 500,000 of compound (2) produced a striking sympathomimetic action on the heart of the cat whereas a concentration fifty times as strong (lower tracing) has no effect on the rabbit's heart. No sympathomimetic action was produced on the rabbit's heart even with solutions so concentrated as 1 in 5000. Compound (3) seems to be the most active of the three on the cat's heart, a quite considerable degree of augmentation and acceleration occurring with a solution of 1 in 2,000,000.

Compound (4).

Dimethoxy-phenylethylamine was found to have no sympathomimetic effect on the heart either of the rabbit or of the cat. Fig. 3 shows the effect of a solution of 1 in 10,000 on the cat's heart. This produced slowing and depression of the heart well manifested in two minutes. When the amine solution was replaced by Locke's solution (L.S.) the heart rapidly recovered and in four minutes had almost returned to its normal rate and amplitude of beat.

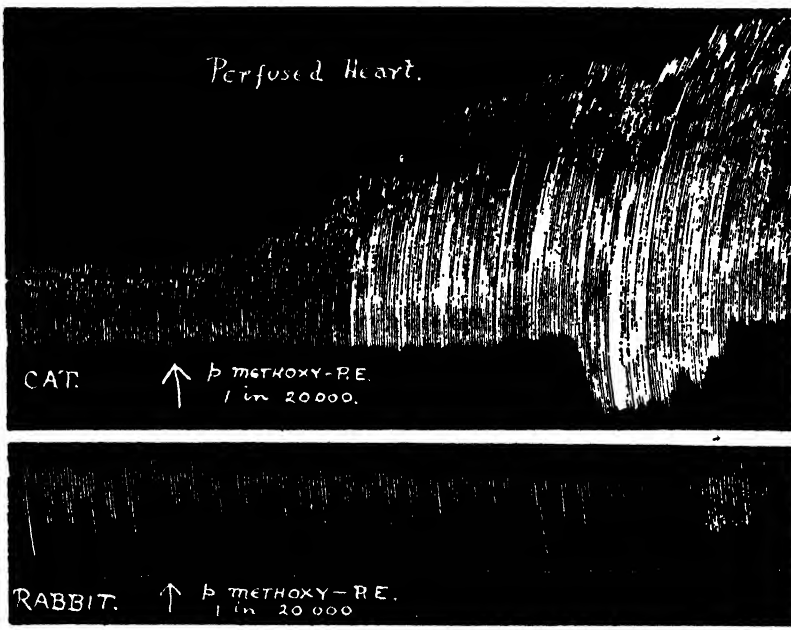


Fig. 1. Isolated perfused mammalian hearts: *p*-methoxy-phenylethylamine 1 in 20,000. Showing sympathomimetic action in the cat (upper tracing) but not in the rabbit (lower tracing).

Action on the coronary vessels.

Adrenaline is known to dilate the coronary vessels, though this effect cannot readily be demonstrated unequivocally in the perfused heart, because it is difficult to determine to what extent a change in the rate of flow is due indirectly to alterations in the heart's contractions. In most of our experiments on the perfused heart we recorded the coronary flow by Gunn's syphon recorder [Gunn, 1926]. In Fig. 2 it can be seen that compound (2) in a concentration of 1 in 500,000 produces a very great

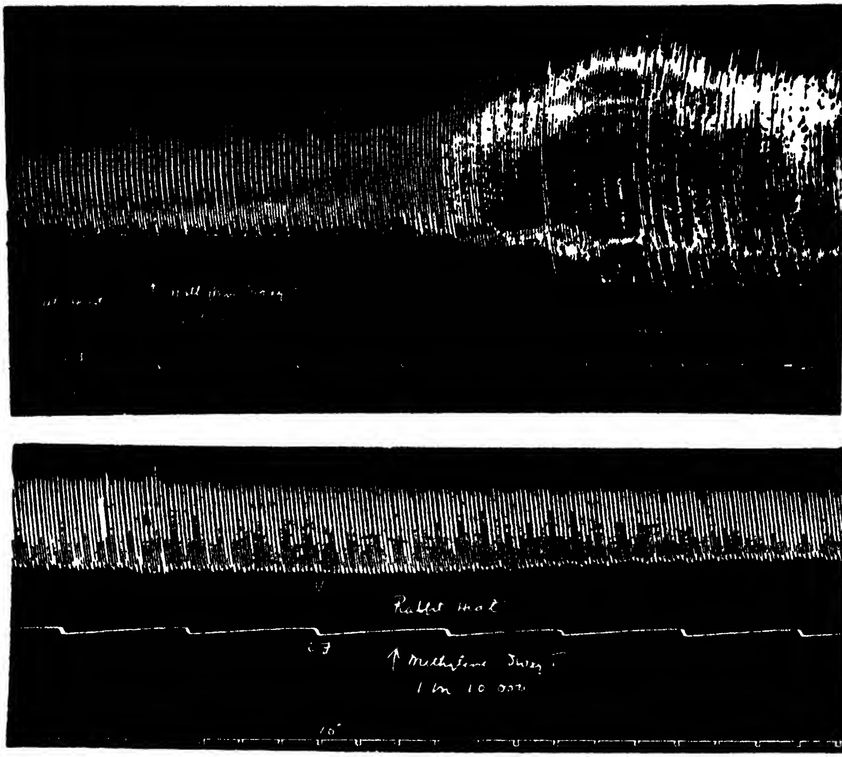


Fig. 2. Upper tracing $= a$, lower tracing $= b$. Isolated perfused mammalian hearts: showing sympathomimetic action of methylene-dioxy-phenylethylamine, 1 in 500,000, on the cat's heart (a), and absence of this effect even with 1 in 10,000 on the rabbit's heart (b). $C.F.$ = coronary flow.

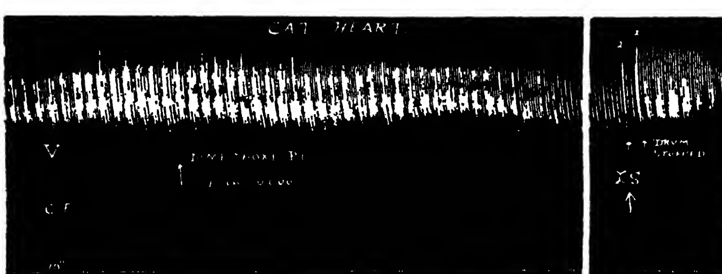


Fig. 3. Isolated perfused cat's heart: dimethoxy-phenylethylamine 1 in 10,000. Showing absence of sympathomimetic action with this compound on the cat's heart.

increase (about 500 p.c.) in the rate of flow through the coronary vessels of the cat, whereas a concentration fifty times as strong has no effect on the flow through the heart of the rabbit.

Fig. 4 shows that increased rate of coronary flow is not dependent on the changes in the heart's contractions. A solution of 1 in 200,000 of the meta compound produced an increase in the rate of flow of about 400 p.c., which could hardly be explained by the relatively slight augmentation of beat. The latter explanation was also negatived by the effect of replacing the amine solution by Locke's solution, because this produced a reduction in the coronary flow in spite of still continued and even enhanced aug-

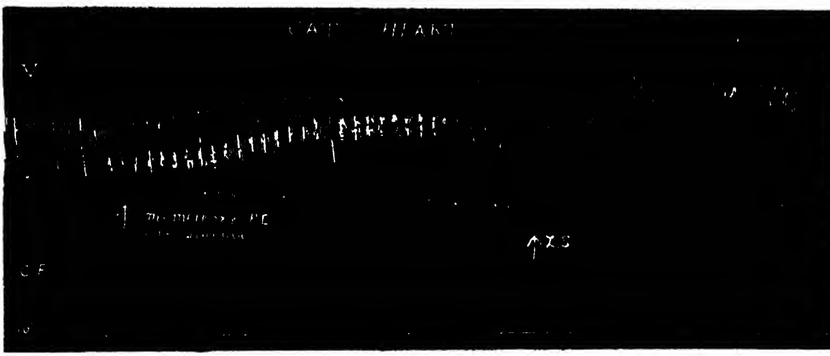


Fig. 4. Isolated perfused cat's heart: *V.* = contractions of ventricle, *C.F.* = coronary flow, each notch representing 2 c.c. Showing sympathomimetic action on the heart, with increased flow through the coronary vessels, produced by *m*-methoxy-phenylethylamine, 1 in 200,000.

mentation of beat. The stimulant effect on the heart of the cat produced by compounds (1), (2) and especially (3) was very prolonged and often continued for many minutes after the solution was replaced by Locke's solution. The effect on the coronary vessels passed off much more rapidly. There was also, particularly with compound (3), a noticeable delay (*vide* Fig. 2 *a*) in the onset of the stimulant effect, as compared with the sudden effect of adrenaline. This delay in action was observed also in the case of the other isolated organs.

From these experiments on the isolated heart one could not evade the conclusion, which was far from expected, that compounds (1), (2) and (3) stimulated the sympathetic in the heart of the cat but not of the rabbit, while compound (4) had no sympathomimetic effect on the heart of either species. The results both on the heart itself and on the coronary vessels supported this conclusion.

Action on blood-pressure.

(*a*) *Cat.*

A large number of experiments were made on cats (decapitated) not only to determine the nature of the effects produced by the amines under consideration, but also to obtain some idea of the quantitative relationships between their activities and that of adrenaline.

Fig. 5 attempts to give an epitome of these results. The method of obtaining this tracing may possess some novelty. The original records

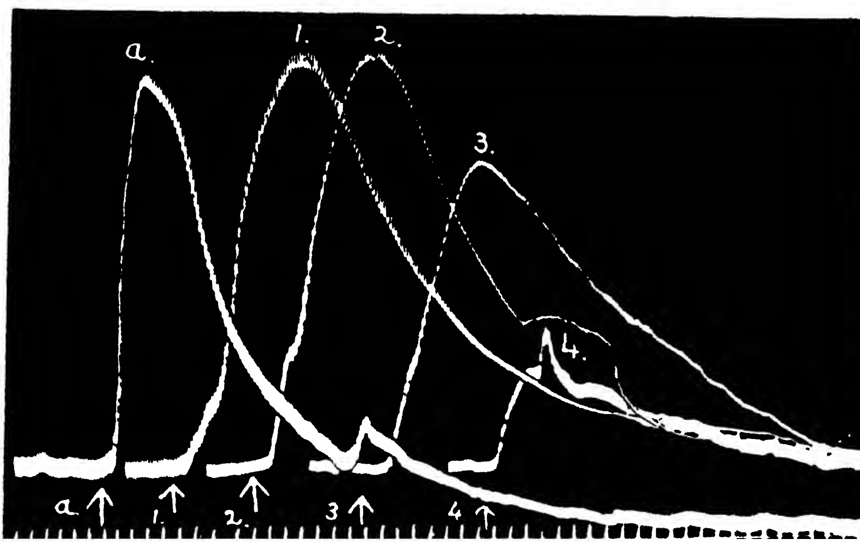


Fig. 5. Record of blood-pressure in decerebrate cat: showing comparative effects on blood-pressure of the following compounds in mg. per kg.: adrenaline 0·015 mg.; (1) *p*-methoxy-phenylethylamine 4·0 mg.; (2) *m*-methoxy-phenylethylamine 4·0 mg.; (3) methylene-dioxy-phenylethylamine 4·0 mg.; (4) dimethoxy-phenylethylamine 4·0 mg. Compounds (1), (2) and (3) resemble adrenaline in action qualitatively.

were taken on a continuously revolving drum in the usual way. The tracings giving the effect of each drug were subsequently photographed separately on half-plate negatives, but at graded distances from the left edge of the plate. The five plates were then placed one over the other and a print taken through all five. The negatives were of course all taken to the same scale. We are indebted to Mr E. G. Long for making this print according to instructions.

Table II gives the actual data. The corresponding figures for the rabbit are also given and will be referred to later.

TABLE II.

Drug	Dose per kg. in mg.	Cat: Rise of blood-pressure in mm. Hg	Cat: Duration of rise in minutes	Rabbit: Rise of blood-pressure in mm. Hg
Adrenaline	0·015	140	2½	88
Compound (1)	4·0	145	4	20
Compound (2)	4·0	150	4	—
Compound (3)	4·0	128	7	15
Compound (4)	4·0	50	3½	30

The para and meta compounds produced, in equal doses, rises of blood-pressure almost equal in height and duration. In this respect they possess at least 1/300 of the activity of adrenaline and the effect is rather more prolonged. The methylene compound produced a smaller but more lasting rise of pressure.

Compound (4) produced a comparatively small rise of pressure with a curve different from that of the others. A second injection of this compound, of 16 mg. per kg., produced a very transient rise of pressure of 30 mm. followed by a prolonged fall.

One experiment of the type described might not necessarily give comparable quantitative results because the previous injection of one or more drugs might render the animal less sensitive to the action of subsequent injections. Many such experiments have been done, however, in which the order of drugs injected has been different and the results were not substantially modified. From several experiments the average ratio of activity of these compounds was as follows: the para and meta compound had about 1/300, and the methylene compound 1/400, of the activity of adrenaline on the blood-pressure of the cat, and the type of pressure curve produced by them was entirely compatible with the view that they stimulate the sympathetic.

As in the case of the heart and coronary vessels, the dimethoxy compound (4) did not produce a typical sympathomimetic effect. It produced in small doses a small rise of pressure.

Larger doses produced a temporary rise of pressure followed by a fall. Increase of dose beyond a certain point did not produce a further rise of pressure. In this respect also, it differed from the other three compounds. The secondary fall of blood-pressure produced in the cat by compound (4) bears at any rate a superficial resemblance to that pro-

duced by histamine. Fig. 6 (*a*) shows the preliminary rise and secondary fall of blood produced in a decapitated cat by the dimethoxy compound. This fall lasted several minutes. Six minutes later the same dose of the methylene compound was given (*b*), which produced the usual rise of pressure. Later histamine 1 mg. per kg. produced a characteristic fall of pressure (*c*).

This experiment shows that, when the main effect of (*a*) is a fall of blood-pressure, (*b*) given subsequently still produces a rise of pressure. Those compounds, therefore, act in different ways. The rise of pressure in (*a*) is due to a stimulation of arterial muscle and the subsequent fall in some ways resembles that produced by histamine (*c*).

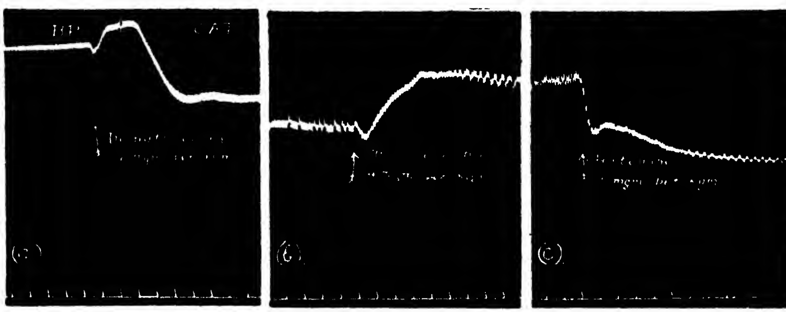


Fig. 6. Record of blood-pressure: showing (*a*) transient rise, followed by a fall, of B.P. by dimethoxy-phenylethylamine; (*b*) rise of B.P. by methylene-dioxy-phenylethylamine; and (*c*) fall of B.P. by histamine.

(*b*) *Rabbit.*

If the hypothesis (that the first three compounds stimulate the sympathetic in the cat but not in the rabbit, while compound (4) stimulates the sympathetic in neither species) drawn from experiments on the isolated heart were correct, then it would be reasonable to expect that there would be corresponding differences in the effects of the first three compounds on the blood-pressure of the cat and rabbit. This was found to be the case. In Table II are compared the effects of similar doses per kg. on the cat and rabbit. The rabbits were anæsthetized by urethane and had both vagi cut, so as to remove cardiac inhibitory influences which are also absent in the decerebrate cat. The table shows that, under these conditions, a given dose of adrenaline produces a smaller rise of pressure in the rabbit than in the cat. It also shows a complete lack of correspondence between the effects of equivalent doses of the amines under consideration on the blood-pressure of the two species. Thus a dose of the

para compound (4 mg. per kg.) which, in the cat, caused a rise of blood-pressure equal to that produced by 0·015 mg. of adrenaline, caused, in the rabbit, less than 25 p.c. of the rise produced by the same dose of adrenaline. Moreover a dose of the dimethoxy compound, which caused in the cat a rise of pressure of one-third of that produced by the para compound, caused a greater rise than the latter on the blood-pressure of the rabbit.

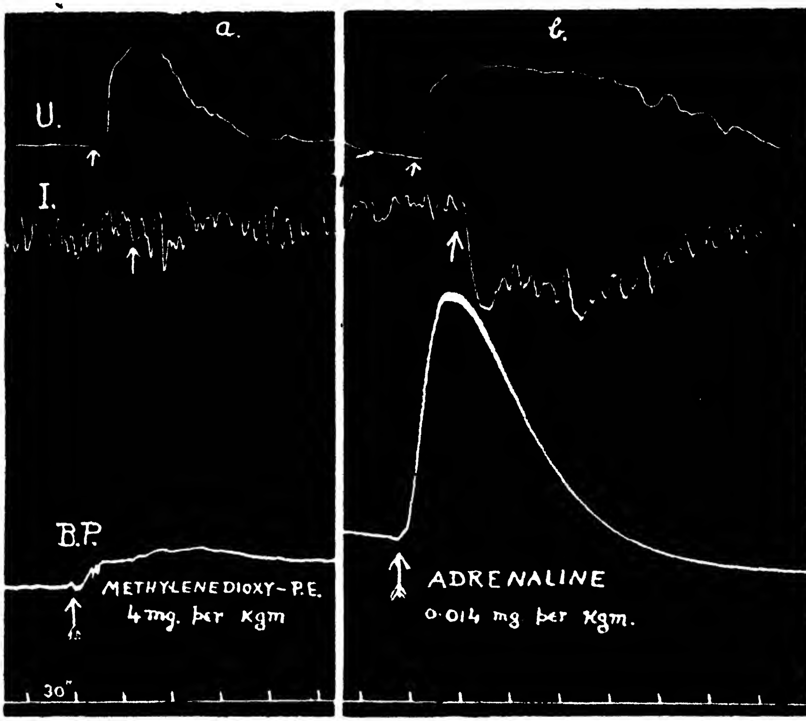


Fig. 7. Records of uterine movements (*U.*), intestinal movements (*I.*), contractions recorded upwards, and blood-pressure (*B.P.*) in a rabbit, anæsthetized by urethane (vagi cut). Showing (*a*) that methylene-dioxy-phenylethylamine does not produce sympathomimetic effects in the rabbit. The intestine is not relaxed or the B.P. raised as by adrenaline (*b*).

In the case of all four compounds, no greater rise of pressure was produced by increasing the dose. The rise of pressure was relatively insignificant and bore little resemblance to a sympathomimetic effect. This is illustrated in Fig. 7. In this experiment the same doses were given as in Figs. 5 and 8, but the effect of the methylene compound in the rabbit is inconspicuous as compared with the effect in the cat. This difference is

also shown by comparison with the same dose of adrenaline in each species.

Experiments on blood-pressure, like those on the isolated heart, suggest, therefore, that the first three compounds stimulate the sympathetic in the cat but not in the rabbit. In the rabbit they produce a slight rise of blood-pressure, such as might be produced by a weak stimulation of arterial muscle. The latter type of effect is produced by the dimethoxy compound in both species.

Additional evidence as to the presence or absence of sympathomimetic action was obtained from experiments on other types of smooth muscle, viz. uterus, intestine, bronchi and pupil.

Uterus.

The uterus affords a convenient test for sympathomimetic action as the nature of its response to sympathetic stimulation varies with different species. Experiments were done on the uterus both *in situ* and isolated.

In the former case the anæsthetized animal was immersed up to the thorax in a saline bath at body temperature; the abdomen was opened, the uterus at some point near the vaginal end was attached to a rigid rod, and a hook, inserted in the uterus nearer by an inch or more to the ovarian end, was attached by a thread to a lever which recorded the uterine movements. In some experiments the movements of the intestine were similarly and simultaneously recorded. A record was also taken of the blood-pressure.

Cat. In the anæsthetized or decapitated animal, compounds (1), (2) and (3) produced a relaxation, and inhibition of movement, of the non-pregnant uterus, identical with that produced by adrenaline. This is illustrated in Fig. 8.

The isolated non-pregnant uterus was also inhibited by these compounds, in weaker concentrations (*e.g.* Fig. 9 *a*). Stronger solutions, however, sometimes caused contraction of a non-pregnant uterus which was inhibited in the usual manner by adrenaline. There was considerable variation in different uteri as to the concentration which produced relaxation or contraction.

Compound (4) produced contraction of the isolated non-pregnant cat's uterus even when all other three compounds caused relaxation. This is shown in Fig. 9 *b*. In this experiment compounds (1), (2) and (3) produced relaxation of the uterus in all concentrations up to 1 in 10,000. The

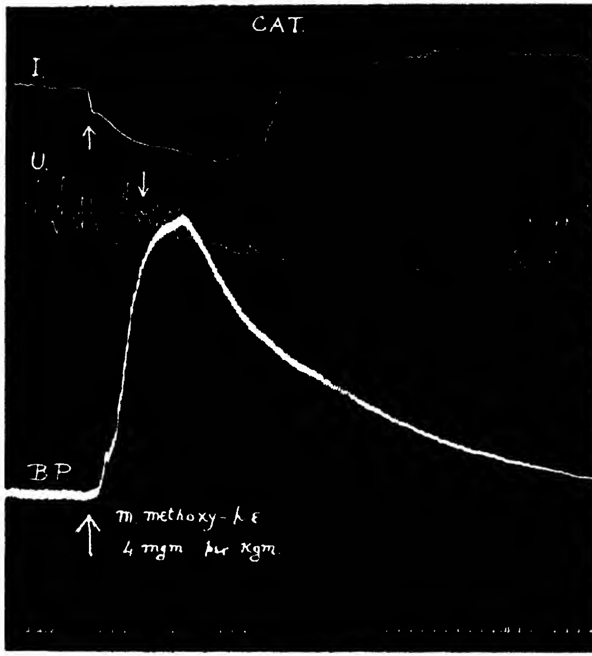


Fig. 8. Record of intestinal movements (*I.*), uterine movements (*U.*), contraction recorded upwards—and blood-pressure (*B.P.*) in a decapitated cat. Showing typical sympathomimetic effects, relaxation of non-pregnant uterus and of intestine and rise of blood-pressure. Contrast the effects in the rabbit, Fig. 7.

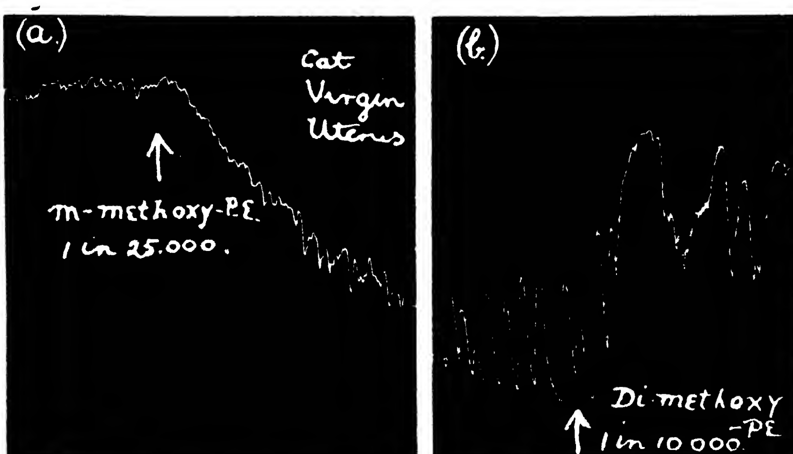


Fig. 9. Isolated virgin cat's uterus. Showing relaxation (sympathomimetic) action of *m*-methoxy-phenylethylamine, and (*b*) contraction (direct stimulation of muscle) by dimethoxy-phenylethylamine.

dimethoxy compound, however, produced a slight contraction with 1 in 25,000 and a marked contraction with 1 in 10,000.

The most obvious conclusion, and one which is supported by other evidence, to be drawn from experiments on the cat's uterus is that the first three compounds have a double action, (*a*) a stimulation of the sympathetic, which is shown in the intact animal and by weaker concentrations on the isolated organ, and (*b*) a direct stimulation of the uterine muscle, especially produced by higher concentrations, which marks the inhibitory effect of sympathetic stimulation. Factor (*a*) is weak or absent in the case of compound (4), which only exhibits the stimulant action on muscle.

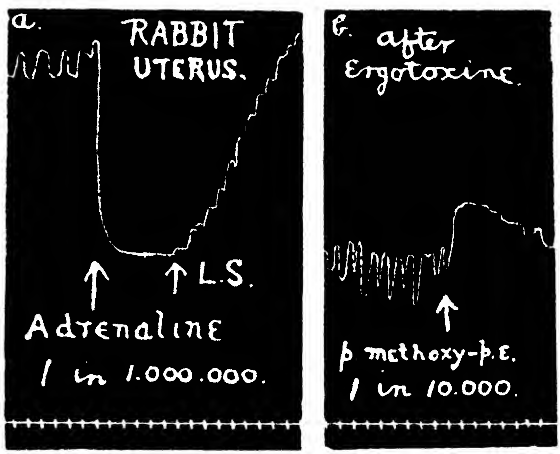


Fig. 10. Isolated rabbit's uterus. Ergotoxine, previously added, converted a motor effect of adrenaline into an inhibitor effect (*a*), but *p*-methoxy-phenylethylamine (*b*) still produced a motor effect (direct stimulation of muscle).

Rabbit. All four compounds stimulated the uterus *in situ* or isolated, of the rabbit (*vide* Fig. 7). As in this animal the effects of sympathetic stimulation and of direct stimulation of uterine muscle are identical, a motor response might be due to either action. A differentiation between the two possible actions could, however, be made by means of ergotoxine. In the experiment which is illustrated in Fig. 10, the isolated rabbit's uterus responded to adrenaline, 1 in 1,000,000, by a powerful contraction. Ergotoxine, 1 in 100,000, was then added to the bath and, subsequently, the same concentration of adrenaline produced a marked relaxation of the uterus (*a*). According to the accepted explanation, ergotoxine had paralysed the motor sympathetic terminations of the uterus. But the

para compound, as shown in the figure, still produced a pronounced contraction of the uterus (*b*). This contraction must, therefore, have been due to a direct stimulation of the uterine muscle.

Guinea-pig. The uterus of the guinea-pig, whether pregnant or non-pregnant, is relaxed by sympathetic stimulation. All four compounds, however, in whatever concentrations they were employed, caused contraction of the guinea-pig's uterus. This is shown in Fig. 11, where both

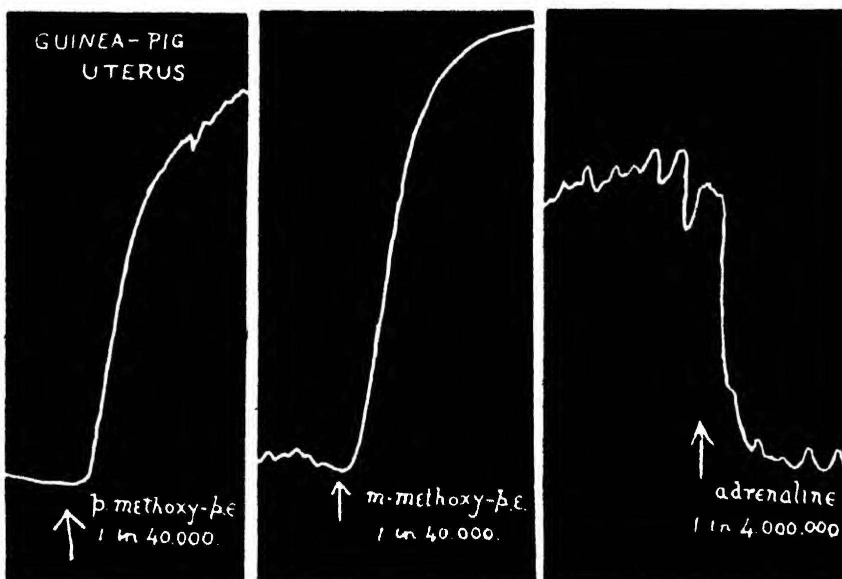


Fig. 11. Isolated guinea-pig's uterus. Showing difference in action between methoxyphenylethylamines (direct stimulation of muscle) and adrenaline (stimulation of sympathetic).

para and meta compounds in a concentration of 1 in 40,000 caused contraction of the uterus, while adrenaline 1 in 4,000,000 caused the usual inhibition.

Rat. The uterus of the rat, which is also inhibited by adrenaline, was stimulated by all four compounds.

It is evident from these experiments that, in rodents, all four compounds have a stimulant action directly on uterine muscle and that there is no evidence of true sympathomimetic action with any of them.

Intestine.

The intestine, isolated or *in situ*, of the *cat* was inhibited by compounds (1), (2) or (3), as is illustrated in Fig. 8. Strong solutions, *e.g.* 1 in 5000, sometimes produced some increase of tonus of the exsected gut. The action on intestinal muscle, therefore, corresponds with that on the uterus, *i.e.* the effect of weaker concentrations is sympathomimetic, but a direct stimulant action on muscle may occur with strong solutions. In the *rabbit* or *guinea-pig*, however, the effect of all four compounds was to increase the tone or movements of the gut. A slight effect of this nature is seen in Fig. 7 *a*, which contrasts with the pronounced relaxation of the gut produced in the cat (Fig. 8). Larger doses produced a powerful contraction of the rabbit's intestine. The inhibitory effect of adrenaline itself on the intestine muscle is also more pronounced in the cat than in the rabbit.

Bronchi.

Experiments were made on isolated bronchial and tracheal rings. The results were of the same type as had been found with other forms of smooth muscle. In the cat compounds (1), (2) and (3), but not (4), caused a relaxation of the bronchi, but in the rings of the rabbit they failed to produce this effect.

As one of the original objects of this research was to attempt to discover a compound which would have the same action on the bronchi as adrenaline or ephedrine but would be intermediate between these two in duration of effect, a typical experiment may be described in greater detail.

As has been found by previous investigators, experiments on the bronchi, exsected or *in situ*, may fail to reveal a sympathomimetic effect (dilator) owing to the muscle of the bronchi being already in a state of maximum relaxation. We found, as others have done, that it was necessary first to give some drug which would produce a degree of bronchial constriction in order that a dilator effect could manifest itself. For this purpose we used arecoline in low concentrations.

Fig. 12 illustrates one such experiment. Arecoline, 1 in 3,000,000, was first applied and produced a marked contraction. Methylene-dioxyphenylethylamine 1 in 10,000 was then added to the bath and, in spite of the continued action of arecoline, produced a rapid and marked relaxation. The alkaloidal solutions were then replaced by Locke's solution and, after an interval, the experiment was repeated with the substitution of

ephedrine for the methylene compound. The curves of the two experiments are remarkably similar and they suggest that, so far as the cat is concerned, the methylene compound acts on the bronchi very similarly to ephedrine.

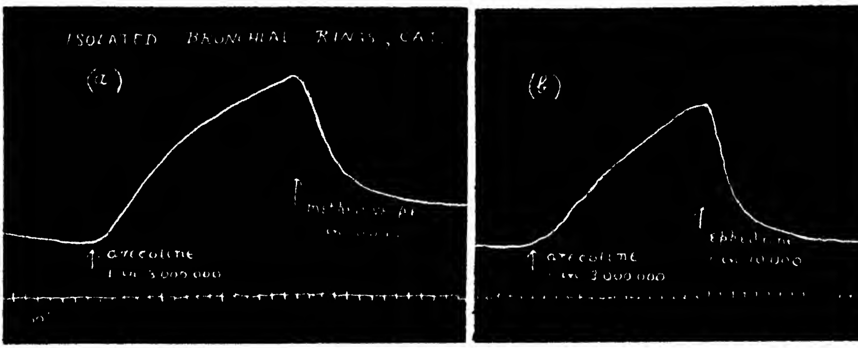


Fig. 12. Isolated bronchial rings of cat. Showing (*a*) relaxation by methylene-dioxyphenylethylamine of bronchi, previously constricted by arecoline. This compound acts like ephedrine (*b*).

Pupil.

In the cat, sympathetic stimulation, or injection of adrenaline, produces, among other effects on the eye, dilatation of the pupil and withdrawal of the nictitating membrane. Compounds (2), (3) and (4) were tested on a cat, anæsthetized with ether, to see whether they produced these effects. Compounds (2) and (3) in doses of 4 mg. per kg. produced withdrawal of nictitating membrane and wide dilatation of the pupil, but the same dose of compound (4) produced neither of these effects. This affords further confirmation for the view that compound (4) has no sympathomimetic action in the cat, corresponding to that produced by the other three compounds.

Table III gives a summary of the actions of the four compounds. It shows that in the cat the first three compounds reproduce with complete fidelity both the motor and inhibitor effects of adrenaline. In rodents, however, they produce effects which do not correspond to those of adrenaline. Especially the characteristic cardiac effects are absent and the inhibitory effects on smooth muscle are absent or replaced by stimulation. The effects in rodents are characteristic of a direct stimulation of smooth muscle.

Compound (4), on the other hand, has actions which do not correspond

with those of adrenaline even in the cat, but are typical of a stimulation of smooth muscle in all animals.

TABLE III. Comparison of action of different compounds on different tissues in different species.

[+ = contraction; − = relaxation; 0 = no effect in equivalent doses.]

	Adrenaline	Compounds (1), (2) and (3)	Compound (4)
Cat:			
Blood-pressure	+ +	+ +	+
Heart	+ +	+ +	0
Coronaries	−	−	0
Uterus (n.p.)	−	−	+
Intestine	−	−	+
Bronchi	−	−	0
Pupil	−	−	0
Rabbit:			
Blood-pressure	+ +	+	+
Heart	+ +	0	0
Uterus	+	+	+
Intestine	−	+	+
Bronchi	−	0	0
Guinea-pig and rat:			
Uterus	−	+	+
Intestine	−	+	+

DISCUSSION.

The results of these experiments seem clear-cut and allow certain conclusions as to the relation between chemical constitution and physiological action in the particular group of compounds under consideration.

Methylation of phenolic hydroxy groups in phenylethylamine compounds modifies the actions of the resulting compound on the central nervous system, on the sympathetic nervous system, and on involuntary muscle, in connection with which the following points may be of some general interest.

Central nervous system.

Methylation of a single —OH group in the para position changes the action from depressant to convulsant. A similar type of change has been described by Gunn and his co-workers in connection with the harmine alkaloids, for whereas harmol and harmalol, which possess an —OH group, are non-convulsant, harmine and harmaline, which differ from harmol and harmalol only in the methylation of this —OH group, are both convulsant [Gunn and MacKeith, 1931].

The meta-methoxy- and methylene-dioxy compounds also stimulate the central nervous system, but the action of the dimethoxy compound

is almost purely depressant. A scrutiny of the effect of methylation of hydroxyl groups (as a single change) on the action of different alkaloids on the central nervous system may, if sufficient data be obtained, lead to the possibility of some general rule being applicable to the physiological effect of this change in chemical constitution. In the meantime it is clear that relatively slight changes in the benzene nucleus of this type of compound, without alteration of the ethylamine side chain, can effect conspicuous alterations in the action on the central nervous system.

Methylation of an —OH group does not necessarily lead to diminution of general physiological activity for *p*-methoxy-phenylethylamine is, by intraperitoneal injection, five times as toxic as *p*-hydroxy-phenylethylamine.

"*Sympathomimetic action.*"

The investigation of the actions of these compounds on the sympathetic system has led to some unexpected results. Para- and meta-methoxy-, and methylene-dioxy-, phenylethylamines all exert typical sympathomimetic actions on the cat but not on the rabbit, guinea-pig or rat. Many amines related to adrenaline produce sympathomimetic effects; but, as a rule, with greater variation from the structure of adrenaline, there occurs greater deviation from consistent or complete duplication of the effects of sympathetic stimulation. Thus, of the amines which have been more completely investigated, tyramine and ephedrine produce many effects which are definitely "sympathomimetic" but produce other effects on smooth muscle which cannot be explained simply by stimulation of sympathetic terminations. We hope in a future paper to reconsider some of the actions of tyramine and ephedrine in the light of the results obtained with the compounds dealt with in this paper. It may be suggested, however, that hitherto not sufficient attention has been paid to differences in reaction due to differences in the species of animal used. It has been usual to state that an amine has sympathomimetic actions on one organ and not on another. It would seem *a priori* more probable, however, that the "receptor" substance for any particular amine should be either present or absent in all the organs of a particular species of animal than that it should be present in one organ, and absent in another organ, of the same species of animal. In other words, it would seem more reasonable to expect that, if a substance stimulates the sympathetic terminations at all, it would stimulate them throughout in the same animal. This certainly seems to be true of the first three compounds in our series. Any one of them could (though they would be less active)

act as a sympathetic hormone in the cat but not in rodents. Adrenaline, on the other hand, seems to be an almost universal sympathetic stimulant for all species of mammals. It is, however, possible, and the possibility has not perhaps yet been excluded, that the adrenal hormone may not be in all species of animals adrenaline itself but some near relative of it.

That dimethoxy-phenylethylamine should fail to display sympathomimetic action even in the cat is a surprising result, especially in view of the fact that adrenaline has two —OH groups in the same position as the two methoxy groups in our compound. Some experiments, recently made in this laboratory with trimethoxy-phenylethylamine (mescaline) have shown that it also is devoid of sympathomimetic action. In their investigation of the amines allied to adrenaline, Barger and Dale showed that the optimum condition of the nucleus for sympathetic action was provided by two hydroxyl groups in the benzene nucleus, but that a less degree of sympathetic activity was shown by mono- and tri-hydroxy-phenylethylamines. On the other hand, sympathomimetic activity is only shown by mono-methoxy compounds, and not at all by di- or tri-methoxy compounds. One cannot at the moment hazard any plausible conjecture as to why this should be.

Direct action on smooth muscle.

All the compounds of our series stimulate smooth muscle in rodents. In the case of the first three compounds, this action is, in the cat, usually masked by sympathetic stimulation. The fourth compound is the most powerful direct stimulant of smooth muscle, and this is its only peripheral action even in the cat. In some respects it recalls the action of histamine.

SUMMARY.

The physiological actions of (1) *p*-methoxy-phenylethylamine, (2) *m*-methoxy-phenylethylamine, (3) methylene-dioxy-phenylethylamine, and (4) dimethoxy-phenylethylamine have been investigated.

The minimum lethal dose per kg. by intraperitoneal injection for mice is approximately (1) 0·15 g., (2) 0·23 g., (3) 0·30 g. and (4) 0·42 g.

The first three stimulate, the fourth depresses, the central nervous system.

The first three stimulate the sympathetic terminations in the cat but not in rodents; the fourth has no sympathomimetic action in any of these animals.

In the decerebrate cat, compounds (1) and (2) have about 1/300, compound (3) about 1/400, of the pressor activity of adrenaline.

All four compounds have a direct stimulant action on smooth muscle in rodents.

Methylene-dioxy-phenylethylamine (3) is, in duration of effect, intermediate between adrenaline and ephedrine, and may be worthy of a clinical trial in the treatment of asthma.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

IV. Changes in the adrenal gland of the female rat associated with the œstrous cycle.

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THERE are many isolated bits of evidence that point towards an intimate relationship between the adrenal cortex and the reproductive system. To review the entire literature relating to the subject is too large a task to be undertaken here. The two most suggestive types of evidence are the presence of abnormalities in secondary sex characters in cases of tumours of the adrenal cortex [Glynn, 1921, and others]; and the changes in the adrenal gland associated with various phases of reproduction. The present paper treats of the latter.

Hypertrophy of the cortex has been found associated with pregnancy in guinea-pigs [Castaldi, 1922; Kolde, 1913; Guieyesse, 1899; Kolmer, 1912] and its occurrence in rabbits [Kolde, 1913; Stilling, 1898] and in rats [Herring, 1920; Donaldson, 1924] has been denied. In mice there is diminution of the size of the adrenal, but hypertrophy of the zona glomerulosa [Masui and Tamura, 1926; Tamura, 1926]. The hypertrophy in guinea-pigs has been found associated with mitoses in the cortex and with enlargement of the cells in the zona fasciculata [Kolmer, 1912; Guieyesse, 1899; Castaldi, 1922]. It continues through lactation [Verdozzi, 1914]. A similar hypertrophy has been reported in spayed and castrated animals by Schenk [1910], Kolmer [1912], Kolde [1913], and others. Hatai [1913], working on rats, reported a decrease in the female and an increase in the male adrenal after removal of the gonads.

All observers agree on one point, and that is that the adrenal weight varies greatly in normal animals. According to Donaldson [1915] the only other organ weights in rats weighing 150 g. which vary to the same degree are the gonads. Boycott and Kellaway [1924] suggest that this variability indicates that form and function are less closely related

here than in most organs. However, we are as yet unable to name the function of the cortex and are therefore at a loss to say whether hypertrophy means decreased function as in the thyroid or increased function as in most organs. Marassini [1906] noted a greater variability in the female, and many workers have observed a larger adrenal in the female than in the male.

Enlargement of the adrenal in connection with ovulation has been noted in frogs [Stilling, 1898], moles [Kolmer, 1918], and in pigeons [Riddle, 1923]. In all of these animals the reproductive season is limited to early spring. The first two undergo other marked physiological changes at that time, so that a direct correlation between the adrenal and ovulation as pointed out by Stilling is not clear. The only animal having a more frequent period of œstrus and one uncomplicated by other factors which has been studied is the mouse. Masui and Tamura [1926] reported enlargement of the adrenal cortex during œstrus in the mouse, but give very few data. Howard-Miller [1927] and Deanesley [1928] were unable to confirm it.

Many workers have investigated the effect of bilateral adrenalectomy on the œstrous cycle. The results have been variable, but the majority find a lengthening of the cycle in the adrenalectomized animals which do not have an abundance of accessories. The recent paper by Martin [1932] reviews the previous ones. That this effect is evidence of a specific cortico-gonadal relationship is unproven, as the œstrous cycle is known to be affected by any procedure which severely affects the well-being of the entire organism.

The present work is based on the probability that if adrenal changes can be found in pregnant and castrated animals they may also be associated with changes in the œstrous cycle.

The one property of the adrenal gland which can most readily be noted objectively and treated statistically is its weight, and it was felt that if a definite change in weight were found, one might expect to find also some change in the size or number of cells. Previous data obtained on adrenal weights in male rats convinced us that the relative weight was more constant, and therefore more significant than the actual weight at any given age, and it was therefore used. The rat was selected as the experimental animal because it has a regular cycle unrelated to season and because of the large numbers of animals required in obtaining reliable statistical data on so variable a thing as the adrenal weight.

TECHNIQUE.

In all, 190 female virgin rats of the same breed were used, and all but a few of Series I were bred in the laboratory.

The first series of rats used in this experiment and reported in Tables I and II included 127 animals. The rats were some of those used in an experiment on the effect of thymectomy on the age of puberty. In 20 of those reported in Table I the smears were not made over a long enough period to be suitable for the analysis in Table II. The adrenals, and in some cases the thyroid and pituitary, were weighed, and it was shown that thymectomy had no effect on the weights of these organs [Andersen, 1932]. The data on these thymectomized rats are therefore included. All rats having any evidence of ear or lung infections at autopsy were carefully excluded, as these are known to affect the adrenal weights [Donaldson]. The rats were given a standard synthetic diet, *ad lib.* [Andersen, 1932], with due regard to good light, cage space, and water. Vaginal smears were made daily, beginning with the day on which the vagina opened.

Inspection of the figures obtained from these rats and grouped according to vaginal smears on the day of death showed that although there was a definite difference in the mean absolute and relative adrenal weights at different stages of the œstrous cycle, the individual variation was great, especially during the late post-œstrus and diœstrus phases. The rats with irregular cycles or cycles 6 days or more in length were then placed in a separate group and the remainder were regrouped with relation to the number of days after the first appearance of abundant cornified cells (4+) in the vaginal smear. The rats showing nucleated epithelial cells were included in the "pro-œstrus" group as before. The day after this nearly all the rats had a vaginal smear showing "4+" cornified cells, and this was termed the day of œstrus. On the two days after œstrus the smear varied greatly: there were cornified cells, non-nucleated epithelial cells or leucocytes in varying amounts. The animals were grouped according to the number of days after œstrus instead of by the smear. The rats killed during late diœstrus, when the smear was still of the diœstrus type but the uterus showed early œstrus changes, were assigned to a separate group. The resulting figures gave a much sharper difference and this plan was subsequently followed. The grouping is therefore as follows:

(1) Pro-œstrus, smear showing epithelial cells, uterus much enlarged and filled with fluid.

(2) Œstrus, the first appearance of an abundance of cornified cells, uterus enlarged but containing less fluid.

(3) The day after (2), smear usually showing 4+ cornified cells but sometimes non-nucleated epithelial cells or leucocytes, or any combination of these; uterus rather smaller and contains no fluid.

(4) The day after (3) with a smear showing a few cornified cells or leucocytes; uterus quite small.

(5) Two days after (4). This stage was included only when the rat had regular cycles of 5 or 6 days in length. When the cycles were longer the rats were excluded from this series and when shorter the data were placed under (1). It was therefore necessary to know the usual length of the cycle for each rat, which was fairly constant.

(6) The day before expected œstrus, smear diœstrus; uterus shows early œstrus changes.

Since some of these stages are so short as to be missed in smearing only once daily, the 53 rats of our second series and the 20 rats of our third series were smeared every 8 hours for a period covering at least three complete cycles. The hours chosen were 9 a.m., 5 p.m., and 1 a.m. The rats were killed according to the classification of œstrus given above. The pro-œstrus group was killed immediately after the first appearance of large numbers of nucleated epithelial cells. The "œstrus" group was killed after the first smear, in which many cornified cells were found, usually with many nucleated epithelial cells still present. The other group was killed at a given number of hours after "œstrus": day after "œstrus," after 24 hours; second day after "œstrus," after 48 hours; diœstrus, 60–72 hours; day preceding expected œstrus, at any period after 72 hours, when the uterus showed early œstrus changes and the smear was still diœstrus.

These rats were of the same breed and received the same care as the first series, with the addition of 5 p.c. of dried yeast to the diet, which had no effect on the mean weight of either the body or the endocrine glands which were weighed. The results obtained from the greater frequency of smearing are only a little more clear-cut than in the first series, probably because the mean adrenal weight changes gradually during the cycle. In this second series the rats on the whole were a little older and heavier, but the majority in both series was between 80 and 140 days of age with the actual range 68–170 days. Four rats are added which had had no cycles for many weeks: they were all 8 months old and are not included in any of the other data. A third series included ten rats on a different but adequate diet, which contained 20 p.c. of fat. The

adrenals were serially sectioned and used for data on the cortex-medulla ratio. The weights of the adrenals in Series III are not included in the figures for adrenal weights at œstrus and diœstrus, but they correspond to them quite closely.

The animals were killed with chloroform, and autopsied at once. The desired organs were quickly dissected clean of connective tissue and fat and placed at once in a closed weighing bottle. The lungs and temporal bones were examined for evidences of infection. The organs were then weighed at once to 0·1 mg. It is believed that the error in dissection and weighing of the adrenals and pituitaries is not over 0·5 mg. The error is much greater in the thyroid weights because the outlines of the gland are not clear-cut. Moreover, the parathyroids are embedded in the thyroids and the weight of the thyroid includes them also. The organs were removed from the weighing bottle one by one and the bottle was weighed after the removal of each. In the first series the adrenal was weighed in each case and the thyroid and pituitary in some cases. In the second series the pituitary, thyroid, adrenal, and thymus were weighed. The ovaries and uterus were examined and preserved, but not weighed. After weighing, the organs were placed in Zenker's fluid, except that in some cases one adrenal was placed in 10 p.c. formalin for fat stains.

In a group of 44 rats in the first series the ovaries, uterus, Fallopian tubes, pituitary, thyroid, and adrenal were sectioned. In a group of 16 rats of the second series these organs were serially sectioned. They were stained with hæmatoxylin and eosin. In the latter group frozen sections of one adrenal were made and stained for fat with Scharlach R and Nile blue sulphate. The data on the pituitary and thyroid changes will be presented in a subsequent paper.

In the ten rats of the third series both adrenals were serially sectioned at 5μ and every tenth section was mounted. The sections were stained with hæmatoxylin and eosin and tracings of the outlines of the entire adrenal and the medulla were made with a projection apparatus at a magnification of $\times 40$. These tracings were measured with a planimeter and the volume of the entire adrenal was calculated from the following formula:

$$\text{volume in cmm.} = \frac{\Sigma \text{ planimeter readings} \times 50\mu \text{ (thickness of section)}}{0{\cdot}2 \text{ (factor of planimeter)} \times 40 \text{ (magnification)}}$$
$$= \Sigma \text{ planimeter readings} \times 0{\cdot}625.$$

The volume of the cortex was obtained by subtracting the medulla from the total volume.

In each case the probable error of the mean is calculated from the formula:

$$\text{P.E.} = 2/3 \sqrt{\frac{\Sigma x^2}{N}}.$$

THE VARIATIONS OF THE WEIGHT OF THE ADRENAL GLAND IN RELATION TO THE ŒSTROUS CYCLE.

The first series of 127 normal virgin rats, when grouped according to the presence in the vaginal smear of epithelial cells, cornified cells, leucocytes or no cells at all, gave the figures for the mean relative weight at these various stages which are shown in Table I. The difference between the mean relative weight at œstrus and at diœstrus was not impressive, but in view of the number of animals represented it invited further investigation.

TABLE I. The mean relative weight of the adrenals in various stages of the œstrous cycle grouped according to the type of vaginal smear on the day of death.

	Entire series		Rats with cycles over 6 days in length	
Stage of cycle	No.	Adrenals mg./kg.	No.	Adrenals mg./kg.
Pro-œstrus	8	250	1	193
Œstrus	32	263	4	235
Post-œstrus	29	240	7	236
Diœstrus	58	237	12	206
Entire series ...	127	246	24	219

Inspection of the individual figures showed that many of the low figures were in rats having irregular cycles. After first eliminating the

TABLE II. The mean relative weight of the adrenals grouped according to the number of days after œstrus as well as by smear. Rats with long or irregular cycles are excluded, age 68–150 days, weight 110–182 g.

		Grouped by vaginal smear		Grouped by No. of days after œstrus	
Stage of cycle	Vaginal smear	No.	Adrenals mg./kg.	No.	Adrenals mg./kg.
I. Pro-œstrus	Nucleated E.C.	7	260	7	260 ± 26
II. Œstrus	First day 4 + C.C.	19	283	19	283 ± 28
III. Post-œstrus	Second day of 4 + C.C.	4	239	15	248 ± 24
Day after œstrus	W.B.C. or non-nucleated E.C.	11	251		
IV. Diœstrus—2 days after œstrus	Varies: 4 + C.C., W.B.C. or no cells	10	234	29	233 ± 22
V. Diœstrus—3 days after œstrus	No cells	19	233		
VI. Day before expected œstrus	Very few or no cells Uterus œstrous	13	269	13	269 ± 25
Entire series		83		83	

rats on which records were inadequate, the rats having irregular cycles were separated off and the adrenal weights averaged. The resulting figures left no doubt that this group had a lower mean relative adrenal weight than the group as a whole (Table II). Further inspection of the remaining data showed that in many cases the rats killed on the first and second days after œstrus had no cells in the smear while others had 4+ cornified cells or leucocytes. It seemed possible that the time after œstrus was a more important factor than the type of cells in the vaginal smear. The data were regrouped as described above with the results given in Table II. The figure for œstrus is considerably increased, while that for diœstrus is lowered a little. We conclude on the basis of this finding that changes in adrenal weight with the phases of œstrus are more closely correlated with the number of days after œstrus than with the smear on the day of death.

The weight of the adrenal in relation to body weight.

The figures in Table II show that there is a greater variation in the relative adrenal weight at œstrus than at any other period, and a less variation at diœstrus. This seems strange in view of the fact that the criteria for œstrus are more sharply defined than those of diœstrus. Moreover, it happened that one group of 25 animals in the second series on which we attempted to confirm the conclusions drawn from the first series were somewhat older and heavier than those in the first group. They ranged from 100 to 150 days in age and from 150 to 225 g. in weight. In this series the œstrus adrenal was surprisingly low and the diœstrus weight was a little lower than that in Series I. We were therefore led to collect all of our data on œstrous rats and arrange them by age and weight to see if there were a definite correlation. This correlation was found as shown in Tables III and IV and Fig. 1. The relative adrenal

Table III. Rats killed at œstrus grouped by age.

Age (days)	Mean weight (g.)	No.	Adrenals mg.	Adrenals mg./kg.
Under 80	137	3	45·4	329
81– 90	147	3	40·7	295
91–100	163·1	8	44·6	279
101–110	162·6	7	42·7	269
111–120	159·5	4	42·0	224
121–130	198·3	3	48·0	245
131–140	195·0	3	42·2	218
141–150	188·6	5	48·7	264
151–160	—	0	—	—
161–170	219·8	4	51·9	236
	Total	40		

Table IV. Rats killed at œstrus grouped by weight.

Weight in g.	No.	Mean age (days)	Adrenals mg.	Adrenals mg./kg.
120–139	4	81·8	41·3	318 ± 18
140–159	10	101·4	43·0	287 ± 28
160–179	13	109·2	44·0	257 ± 14
180–199	4	131·2	49·5	258 ± 27
200–219	7	129·7	47·7	225 ± 20
220–249	2	166·0	55·0	235 ± 13
Total	40			

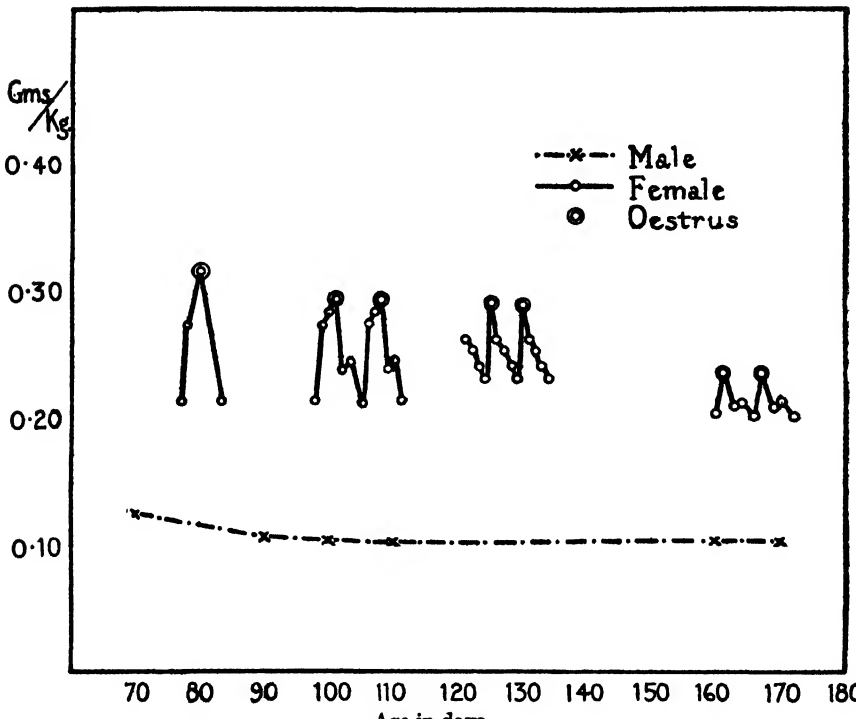


Fig. 1. The relative weight of the adrenal gland at various ages in mature male and female rats.

weight decreases progressively as the weight of the rat increases. This relationship is not so sharply defined in regard to age, although since weight is to some extent a function of age there is some change there. Our experience corroborates Donaldson's tables in regard to the wide variations in weight in normal young adult rats, bred and kept under

conditions which are apparently the same. This table brings out the point that the absolute weight increases gradually with body weight.

These clearly defined results led to rearranging the data for all stages of the cycle according to weight. On the basis of the figures given in Table IV it was decided that all rats under 160 g. would form one group, those between 160–180 g. a second group and those over 180 g. a third group. The results of this rearrangement are shown in Table V. It is

TABLE V. Weight of the adrenals at various phases of œstrus grouped by weight.

Stage of cycle	Body weight: Under 160 g.			160–180 g.			Over 180 g.		
	No.	mg.	mg./kg.	No.	mg.	mg./kg.	No.	mg.	mg./kg.
Pro-œstrus	5	39·6 ± 4·0	286 ± 23	0	—	—	1	37·7	208
Œstrus	14	42·5 ± 3·4	296 ± 27	13	43·9 ± 0·3	265 ± 15	13	49·2 ± 4·3	237 ± 16
First day after œstrus	7	33·0 ± 2·4	240 ± 30	4	43·0 ± 0·5	253 ± 25	0	—	—
Second day after œstrus	19	36·0 ± 3·2	249 ± 23	8	41·4 ± 0·4	244 ± 26	6	42·0 ± 2·4	212 ± 6
Third day after œstrus	7	31·0 ± 3·1	212 ± 18	5	40·2 ± 0·7	235 ± 08	1	41·9	215
Day before expected œstrus	11	37·8 ± 2·8	278 ± 20	2	50·0 ± 4·0	295 ± 21	0	—	—

obvious that the greatest difference between the figures for both actual and relative adrenal weights for œstrus and diœstrus is found in the lighter rats. The adrenal weight at œstrus shows a much greater variation than that in diœstrus, although in both cases it is greater in the young animals.

CORTEX-MEDULLA RATIO.

The relative volumes of the cortex and medulla of the ten rats in Series III are given in Table VI. The rats were killed at phases 2 and 5

TABLE VI. Weight of adrenal cortex and medulla.

	Rat No.	Age (days)	Weight (g.)	Adrenals mg.	Adrenals mg./kg.	Cortex mg./kg.	Medulla mg./kg.
Œstrus	1081 op.	61	129	34·7	270·5	253·7	16·8
Diœstrus	1068 op.	64	149	36·6	245·5	227·3	18·2
Œstrus	1061 c.	61	127	40·0	315·0	296·7	18·3
Diœstrus	1076 c.	62	142	35·8	252·1	235·1	17·0
Œstrus	1073 op.	62	170	44·4	261·5	244·5	17·0
Diœstrus	1079 op.	63	132	31·2	236·3	217·4	18·9
Œstrus	1058 c.	63	150	41·5	276·8	260·7	16·1
Diœstrus	1050 c.	64	149	32·5	218·2	199·6	18·6
Œstrus	974	73	168	45·0	268·0	251·7	16·3
Diœstrus	973	72	161	38·9	242·0	225·4	16·6

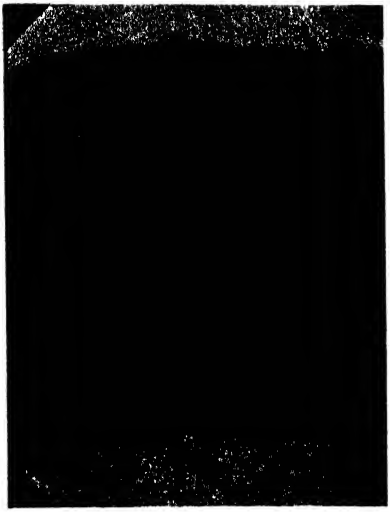
respectively. The mean percentage of medulla was found to be $6{\cdot}08 \pm 0{\cdot}17$ for œstrus and $7{\cdot}51 \pm 0{\cdot}45$ for diœstrus. The mean relative weight of the cortex as calculated from this percentage is $261{\cdot}5 \pm 12{\cdot}2$ mg./kg. for œstrus and $221{\cdot}0 \pm 8{\cdot}3$ for diœstrus. The mean relative weight of the medulla is $16{\cdot}9 \pm 0{\cdot}5$ mg./kg. for œstrus and $17{\cdot}9 \pm 0{\cdot}6$ for diœstrus. It will be noted that the difference in cortex weight for œstrus and diœstrus is significant, while that for the medulla is not, in view of the probable error. This means that the change in weight is due entirely to cortex.

Histological changes.

A comparison of the sections taken from rats killed at œstrus and at 60 hours after œstrus, under the standard conditions given above, reveals no change in the medulla as seen in the hæmatoxylin and eosin or fat stains. There is a change in the cortex (Fig. 2). In diœstrus the adrenal has a wide and clearly defined glomerular zone, outlined by a line of condensed nuclei from the fascicularis. The cells in the outer portion of the fascicularis are somewhat larger than those in the inner portion, but they contain few vacuoles. The cytoplasm is decreased in the inner zone and it merges without any sharp demarcation into the zone of cells bordering the medulla. Near the medulla and often lying adjacent to it are small groups of cells with an eosinophilic cytoplasm which is more abundant than that in most of the cells of the inner third of the cortex.

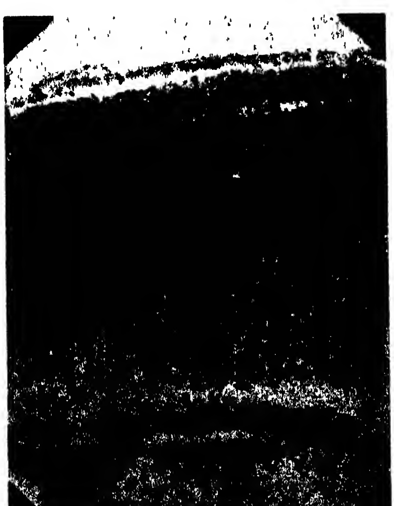
In the adrenal at œstrus the glomerular zone is narrower and less clearly demarcated from the fascicularis. The cells of the outer half or two-thirds of the zona fascicularis are much enlarged, pale and vacuolated. The margin between this area and the more compact cells of the inner third of the cortex is irregular and not sharply defined. The eosinophilic cells near the medulla are enlarged, contain vacuoles and resemble the cells of the outer portion of fascicularis. Mitoses are extremely rare in the adrenal during both œstrus and diœstrus.

Scharlach R stains on frozen sections of the adrenal at diœstrus show an abundant deposit of large droplets of lipoid in the cortex which take the stain deeply. The margin of concentrated cells between the glomerular and fascicular zones and the more compact cells of the inner third of the latter do not take the stain. The nests of cells near the medulla do take it. At œstrus a few cells of the outer fourth of the medulla and some of the cells next the medulla contain large droplets which take the stain, but most of the cortex stains a diffuse pale red. Nile blue stains most of the cortical cells blue, although there is a little red-stained



1. Diœstrus. H. and E.

2. Œstrus. H. and E.



3. Diœstrus. Scharlach R.

4. Œstrus. Scharlach R.

Fig. 2.

material in the inner portion of the zona fascicularis. The blue is somewhat more abundant at œstrus, although the difference is not as striking as in the Scharlach R stains.

The adrenals in animals having irregular cycles or no cycles.

The data given in Table I show that the adrenal weight is quite low in animals having unusually long or irregular cycles. This led us to watch for animals which had had no cycles for some weeks preceding autopsy. Most of these animals were infected and therefore unsuitable for comparison, but four were found which were uninfected, and the adrenals in these animals were found to be quite small, and to have

Table VII. Rats that had no cycles for several weeks preceding autopsy. Ovaries without corpora lutea or large follicles.

Rat	Age (days)	Weight (g.)	Adrenals mg.	Adrenals mg./kg.
43–85	246	165	23	139
45–05	239	159	31	198
46–07	239	196	29	148
46–10	239	187	34	182

approximately the same relative weight as the adrenals of our spayed animals (data unpublished) (Table VII). Whether the lack of ovarian activity or the small adrenal is primary is, of course, still unknown.

Discussion.

The evidence presented points to a definite change in the physiology of the adrenal cortex associated with œstrus. Whether this is an increase or decrease of activity is still a problem. It also remains to be proven that this change signifies a specific causal relationship.

Conclusions.

1. The absolute and relative weights of the adrenal glands of the virgin female rat are greater at œstrus than at diœstrus, providing that the body weight is the same.

2. Rats having had irregular cycles or cycles of 6 days or over are found to have a lesser absolute and relative mean adrenal weight both at œstrus and diœstrus than those with regular cycles.

3. If the relative adrenal weights of the rats killed at œstrus are grouped according to the body weight of the rats, it is found that the absolute weight of both adrenal glands increases with body weight, while the relative weight decreases. The correlation is less close when the grouping is by age.

4. The relative adrenal weight of the rat in diœstrus is a fairly constant figure, regardless of the regularity of the cycle or the weight of the rat and is 0·20–0·23 g. per kg. body weight. The absolute weight is variable.

5. The difference between the mean relative weight of the adrenal gland at œstrus and diœstrus is therefore greater in lighter animals. In our series of 63 animals weighing less than 160 g. and having had regular cycles the mean relative weight at œstrus was 296 ± 27 mg./kg., and at diœstrus 212 ± 18. The difference in relative weight is about 25 p.c. of the figure for œstrus.

6. The difference between the mean absolute weight of the adrenal gland is also greater in lighter animals. The figures for the rats weighing less than 160 g. are: œstrus, 42 ± 3·4 mg.; diœstrus, 31 ± 3·1. This represents a difference of about 25 p.c. of the weight at œstrus, and of 11 mg. between the two means. In the rats weighing 160–180 g. the difference is 4 mg. or about 9 p.c., and in those weighing over 180 g., 7 mg. or about 14 p.c. The mean difference in actual weight is therefore in the same order of magnitude in these series, although the number of rats in the last two groups is not great enough for the actual figures to be final.

7. The increase in weight is found to be due entirely to increase in the size of the cortex. The cells of the zona fascicularis and of the cell nests near the medulla are enlarged during œstrus and contain an increase in lipoid which appears pale pink when stained with Scharlach R.

8. Since the weight and histological appearance of the adrenal of the unmated female rat is so variable it is suggested that, in experiments in which the sex of the animal is a matter of indifference and the weight or appearance of the gland is a criterion, male animals should be used.

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RETROGRADE POLARIZATION, A THEORY OF SYSTEMATIC ERRORS IN MEASUREMENTS OF MUSCULAR CHRONAXIE THROUGH RINGER'S FLUID OR WITH LARGE ELECTRODES[1].

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In the conclusions of a recent paper [Lapicque, 1931*b*] I recalled that chronaxie "reveals a deep modality, an essential property of each particular organization of the living matter"; this assumes that chronaxie depends only on the specific properties of the excitable tissue and not on the way the electric current is applied to it.

This proposition is essential. The various applications of chronaxie have been derived from it. These deductions have been verified by experiment, and thus we might consider the principle verified. But it is worth while to discuss the fact itself, considering only experiments on chronaxie measurements; for it has been contested on this ground, particularly with regard to the size of the electrodes.

In 1926 I was obliged to leave the question open, but I believe I am able to solve it now.

The discussion is related to muscle only; those who oppose my views admit that for nerve everyone is in agreement; though, even in this case, there is an appearance of changes in chronaxie under the influence of some experimental conditions, *e.g.* the chronaxie seems to depend on the distance between the electrodes. It will be interesting to discuss this fact now as an introduction.

We shall first give the experimental facts without using such abstractions as rheôbase and chronaxie. The liminal potential for long durations and the liminal duration for a potential twice as high as the preceding one, do not depend on the distance between the electrodes above 1 cm.; the first increases while the second decreases when the distance between the electrodes is decreased below 1 cm.

[1] Translated from the French by Andrée Monnier-Dumont.

In order to deal with rheobase and chronaxie, the electric current must develop freely its physicochemical action in the tissue, the efficiency of this process on the tissue for stimulation being stopped at a given time by an effect depending on the tissue itself. It is this particular effect which, by its precocity or its delay, characterizes the tissue chronologically, and chronaxie must represent this very characteristic. If, for any reasons, another electrical disturbance hampers the action of the current on the tissue, before the stimulation process is stopped by the normal antagonistic process, we shall evidently miss the information we are looking for in the electric stimulation. For example, if the reverse polarizations which are developed at each electrode come in contact and neutralize each other, the limitation of the effect of the current would depend on something else than the tissue itself, and consequently the chronaxie measured is a false one.

This happens in nerve when the electrodes are only a few millimetres distant. Diffusion, as meant by Nernst (diffusion of a salt in water), would be much too slow to cause each polar perturbation to spread till it came in contact with the other. But the nerve (core conductor) contains in its axis a more conducting central part separated from a less conducting sheath by a polarizable surface. As soon as polarization starts under one electrode it spreads lengthwise, not by the effect of diffusion, but by the action of the current itself which, when opposed by the counter electromotive force that is just created, goes farther and farther to untouched surfaces. This phenomenon, known for almost a century, is the physical electrotonus. Its propagation is comparatively very rapid. From all we know, there is no difficulty in admitting that through this mechanism a notable transmission of positive ions might take place in a thousandth of a second through a centimetre, from the anode to the cathode; that is to say, some neutralization of the cathodic polarization. And this accounts perfectly for the so-called variations of rheobase and of chronaxie according to the variations in the distance of the electrodes.

But such a denomination is evidently inaccurate. There is only one chronaxie: that obtained with a sufficient distance between the electrodes to give the maximum value, which, above this distance, is independent of this experimental condition. This being the case, the value is conditioned only by the properties of the nerve itself and is the chronologic picture of these properties. On the contrary, when the electrodes are nearer to each other, the liminal duration observed is conditioned by a purely physical phenomenon and thus is not a chronaxie. We must not

say "chronaxie decreases with the distance between the electrodes," but "below a certain distance we are dealing, not with a true chronaxie, but with a pseudo-chronaxie deprived of any physiological significance."

The influence of the size of the electrodes on muscular chronaxie measurements offers a problem just the reverse of this one, or better, symmetrical with this one. Let us approach this question as it was presented by Davis [1923]. He says:

> The various workers have been in good agreement as to motor nerve....For striated muscle, however, Lapicque and his school find a chronaxie essentially the same as that of its motor nerve, while Lucas found it approximately ten to twenty times as long. The techniques of the two differ in that Lapicque stimulates the muscle with a small silver electrode, while Lucas used...his fluid electrodes. More recently, Jinnaka and Azuma have applied the pore electrodes...to the study of this problem, and have found values in general agreement with Lapicque...the different values are characteristic of the different techniques. The important variable is the size of the electrode. A small electrode, such as pores from 3 up to 75μ in diameter, or a metal wire, gives short chronaxies, from 0·0002 sec. to 0·0005 sec., while fluid electrodes of the Lucas type give chronaxies up to 0·02 sec. By varying the size of the effective electrode, nearly the whole range of the intermediate values can be covered.

He concludes as follows:

> The pore electrodes resemble a little more closely, in point of size, at any rate, the physiological method of stimulation, *i.e.* by the nerve...but until the laws governing the wide variations can be worked out, it does not seem possible to speak of any single chronaxie as characteristic of a given muscle.

One would suppose that this note, without any numerical result and qualified by the author himself as "preliminary report," would have been followed by a more precise paper. To my knowledge, Davis has not published such a further paper. But a year later, Watts [1924] published a report, evidently a continuation of the work, using even Davis's own electrodes. These were, on the one hand, two Pratt electrodes, *A* with a pore of 3·5μ in diameter, the other *B* with an elliptic pore of 73 × 44μ; and on the other hand, *C*, a fluid electrode of Keith Lucas, with a slot of 5 × 1 mm.

These electrodes being applied on the pelvic end (nerveless) of a frog's sartorius, the following values were obtained as chronaxies, "representative values commonly obtained":

Electrode	Size in mm.	Chronaxie in σ
A	0·0039	0·15
B	0·073 × 0·044	0·55
C	5 × 1	2·7

Another Lucas's electrode with a slot 3·5 times as great—7·5 × 2·5 mm.—gave the same values as *C*; Watts takes no more account of this one, and from the three preceding ones, he concludes that Davis's demonstration of chronaxie as dependent on the size of the pore is confirmed.

At a first glance these figures may be impressive. But, if we consider the constitution of Pratt's and Keith Lucas's [1906] electrodes, of which Watts does not speak, we readily see that the conditions are not homologous.

Pratt's electrodes are made of thin glass tubes which are held perpendicularly to the muscle; the section in contact with the muscle is composed of a comparatively thick wall and of a microscopic hole (pore). The tube is filled with Ringer solution, through which the current is led. This is the cathode. In Watts's experiments the whole preparation, *i.e.* the whole frog with its muscle uncovered and left *in situ*, is immersed in physiological solution, the anode being dipped in this solution.

In Keith Lucas's fluid electrode the muscle is suspended inside a tube with a hole (slot) across its bottom. A more or less long part of the muscle goes through that hole; the part left above is maintained in physiological solution by atmospheric pressure, the top of the tube being closed by a cork, and the electric (negative) current being introduced through a lateral bifurcation. At some distance below this electrode, the inferior part of the muscle touches another physiological solution which constitutes the anode. Consequently, with Pratt's electrodes the current comes in contact with the muscle crosswise at a definite and limited surface. With Keith Lucas's electrodes the current goes lengthwise all around the muscle; near the hole the lines of current converge toward the muscle and penetrate all around it; the surface of penetration is not the surface of the hole, but the product of the muscle circumference by an undetermined length. Thus the two kinds of electrodes are completely different, and it is not justifiable to make a series in which the dimensions of the holes of one of them are compared with those of the other. The two cases must be studied separately.

The glass tubes applied at right angles to the muscle give a definite dimension for the electrodes; with these tubes we shall be able to determine if the dimension of electrodes has any influence on chronaxie.

In all my experiments, until 1930, I had never observed such an influence, but I always used relatively small electrodes, either thin silver wire or small brushes; now, I undertook to find out systematically if

there is any such influence. I found it practical to use my non-polarizable electrodes made of red glass and modified as follows: the end of the tube with the hole has been drawn and curved; by means of a short rubber tube, I could add interchangeable endings made of drawn glass; the free end of these endings is a cross-section, ground smooth on a very fine emery grinder. The dimension of the hole (as well as the thickness of the wall) is measured under a microscope of low magnification ($\times$ 30), with an ocular micrometer previously scaled to an objective micrometer. I prepared a series of such endings with different diameters, varying from 0·08 to 2·5 mm.; the thickness of the walls varied independently, from 0·3 to 1·5 mm. For my experiments I used the sartorius muscle of frogs, generally *R. fusca*, in which the dissection is easier. After the dissection, which must be done very carefully to avoid any injury to the muscle, the preparation is put in physiological solution to rest for about half an hour. Then it is fixed on the bottom of a small trough by two vegetable thorns which are pinned, not through the muscle itself, but in fragments of adjoining muscles left attached to it at each end for that purpose. The electrode described above, manipulated with a screw providing a fine adjustment, is placed at a right angle in the middle of the pelvic end, gently pressed against the muscle surface. The trough is filled with Ringer solution and a silver anode is dipped in it. Chronaxie is measured by means of a chronaximeter functioning by the break of a short circuit, giving in that way measurements within 0·1σ.

Here are the details of the first experiment, which gave already some significant results.

October 26. *R. fusca*; sartorius. Capillary cathode on the middle of the pelvic end. The anode is dipped in the solution, 1 cm. away from the distal end of the muscle.

Electrode *A*: diameter of the hole 0·12 mm., thickness of the wall 1·3 mm.; liminal voltage for 30σ (rheobase) 4·5; with a voltage of 9·0, the threshold is reached when the duration is reduced to 0·4σ.

Electrode *F*: hole 0·66; wall 0·46—rheobase 0·95; for a voltage of 1·9, liminal duration 0·4.

Electrode *B*: hole 0·15; wall 0·52—rheobase 2·2; for a voltage of 4·4, liminal duration 0·4.

The anode is changed to the pelvic side 1 cm. away from the end of the muscle.

Electrode *B*: rheobase 5·0; for a voltage of 10, liminal duration 0·3.

Electrode *A*: rheobase 4·0; for a voltage of 8, liminal duration 0·3.

The capillary electrode is replaced by a silver wire as in the experiments of my preceding papers [Lapicque, 1931]: rheobase 1·9; for a voltage of 3·8, liminal duration 0·7.

The results, within the limits of experimental variation with capillary electrodes, are, as to their liminal duration when the potential is double

that of the rheobase, independent of the instrumental conditions[1]. Thus they correspond to the definition of chronaxie. On the other hand, the value obtained with the silver wire, being about double, shows a slight extra phenomenon, in a way which we shall explain further; we must call this figure a pseudo-chronaxie.

The thickness of wall (the minimum being 1 mm.) has no effect. This may seem surprising when one remembers that chronaxie decreases when the electrodes are nearer to each other. Indeed, here the anode is diffuse around the cathode, whose conductivity is greater than that of the muscle, so that the distance between the point of entrance and of exit of the current (that is to say between the efficient electrodes) varies with the thickness of the wall. We have recalled how in nerve this condition leads to some important consequences. But there is no reason, *a priori*, to find the same physicochemical mechanism in muscle as that based on the existence of a more conducting core. In fact, I do not know any experiment made on muscle on the influence of the distance between the electrodes on chronaxie. It seemed useful, therefore, to take up this point.

We[2] operated on sartorius muscles either immersed in Ringer, or in the air after a long rest in the solution. (The muscle is then wiped before the experiment.) We took two of our capillary electrodes or only one and a silver wire, both being placed on the nerveless region of the muscle, lengthwise, in order to make the current flow in more or less long portions of fibres and not jump from one fibre to another. When we increased or decreased the distance between the electrodes we observed, in some cases, irregular variations which had no relation to the distance between the electrodes. As a rule the result is the same, within the range of experimental error, whether the electrodes are at 6 or 2 mm. apart. If we put them very far apart, 25 or 30 mm., *i.e.* almost across the whole length of the muscle, we still obtain the same figure as for any possible distance within the pelvic end, but we do not then know whether or not we have provoked a nervous stimulation; at any rate, if the electrodes themselves are not able to introduce an α effect, according to a mechanism which we shall explain, the distance between them has no effect on chronaxie.

[1] The variation from 0·4 to 0·3 when the position of the anode is changed is not significant, as the determinations are accurate only to within a tenth of a σ. However, the temperature having risen slightly during the course of the experiment, there might be actually a slight decrease in the chronaxie, at any rate, not correlated with the direction of the current.

[2] Every experiment has been performed with Madame Lapicque.

Thus it is no longer surprising that the thickness of the wall showed no influence.

Let us go back now to our main question: that is the influence of the diameter of the conducting electrode in its insulating sheath, in other words, the inside diameter of our capillary endings.

All the following experiments have confirmed the first one, and they emphasize a general result which we can formulate in this way. From the smallest diameter used, one-tenth of a millimetre, to five or six-tenths of a millimetre, the size of the electrodes has no influence whatsoever.

But above six or seven-tenths of a millimetre the minimal duration for a potential twice that of the rheobase increases regularly with the diameter of the ending. This increase, already conspicuous for a diameter of 1 mm., reaches several σ for a diameter of 2·5 mm.

Here are the figures of a few experiments given in the order they were made; in each couple of figures, the first one represents the diameter of the hole in mm.; the second, in heavy type, the duration found in thousandths of a second.

October 27: 0·36, **0·4**; 0·15, **0·3**; 0·66, **0·3**; 1·5, **1·0**; 0·66, **0·4**; 2·0, **1·5**; 1·0, **0·6**; 2·5, **3·0**.

October 28: 0·66, **0·5**; 0·24, **0·3**; 0·15, **0·3**; 1·5, **1·3**; 2·5, **2·8**; 0·45, **0·4**; 1·0, **0·7**.

November 3: 0·36, **0·5**; 1·0, **0·6**; 0·15, **0·55**; 2·5, **2·5**; 1·0, **0·6**; 0·80, **0·55**; 0·15, **0·50**; 0·08, **0·45**.

November 14: 0·15, **0·3**; 0·30, **0·3**; 0·40, **0·3**; 0·90, **0·5**; 2·5, **1·2**.

These values plotted graphically (abscissæ: diameters of the electrode; ordinates: liminal duration for twice the rheobase) outline systematic curves, each one tending, when starting from large sizes, distinctly toward a constant value which is practically reached between 1 and 0·5 mm. For smaller sizes the variations obtained in our measurements are within the range of experimental error.

We have not in fact used any diameter smaller than about one-tenth of a millimetre. Watts used a much smaller one, of the order of a thousandth of a millimetre, but the values he obtained are hardly smaller than ours; the author gives 0·15σ as a representative figure, but his experimental figures range from 0·12 to 0·35σ. Furthermore, Jinnaka and Azuma [1922] who were the first to make measurements with Pratt's electrodes, found, with a diameter of 10μ, chronaxies of 0·2 to 0·3σ for the sartorius. Thus, from the experimental point of view, it is not likely

that the curve, after it has reached a constant value for diameters of a few tenths of a millimetre, should present a new decrease for much smaller diameters.

Outside the range of constant values, the important fact is an ascending slope of the curve toward large diameters, but, as we noticed that for 2·5 mm. we obtained figures around 3σ, it is worth while to recall values obtained with the bath constituting a "fluid cathode" when the length of the muscle exposed to the current is progressively reduced. The pseudo-chronaxie decreases from 20σ for half of the muscle

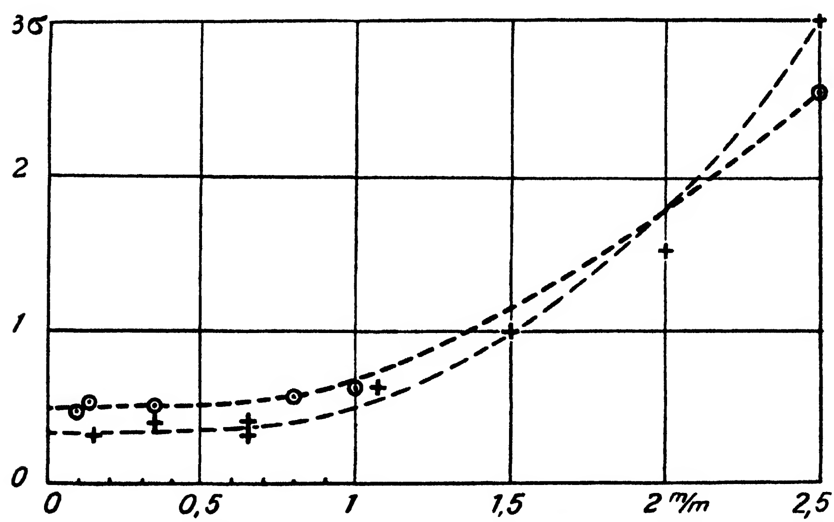

Fig. 1. Two experiments, each one demonstrating the pseudo-chronaxie (as ordinate), raising above the chronaxie (on the left side) according to the diameter of electrode (as abscissa).

to 3σ for 2–3 mm. [Lapicque, 1931 *b*, p. 220]. In other words, the capillary electrode on one side and the solution as used by Rushton on the other give the same chronological characteristic for stimulation when the same length of muscle undergoes the action of the current through the Ringer. The two series of measurements join each other within the limits of experimental error; Fig. 2 illustrates this point. Thus it may be quite possible that one and the same phenomenon is involved for the ascending part of the curve with one or the other apparatus. I previously called this phenomenon the α effect. Every chronological characteristic located on the ascending branch of the curve or on the part which joins it with the horizontal branch, must be, for one and the

same reason, called a pseudo-chronaxie. The horizontal branch is the only location of true chronaxies, that is to say, independent of instrumental conditions, but determined by the muscle's own properties.

There is not only an abstracted, a theoretical limit when the size of the electrodes tends toward 0, as Davis thought. There is, practically, a notable range of dimensions where this value is not a function of the calibre of the electrodes. This circumstance is convenient; from the practical point of view, we do not need to take into consideration the calibre, if we keep it below a given dimension, and that is easy to estimate

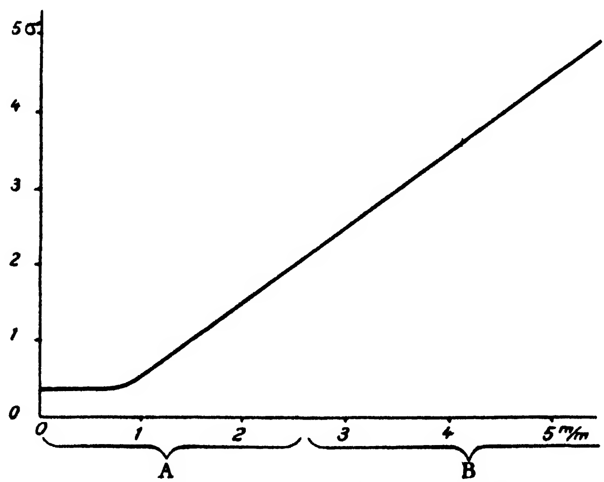

Fig. 2. Schematic graph, to show how experiments with electrodes of varying sizes (in *A*) join experiments in bath of varying lengths (in *B*). Chronaxie or pseudo-chronaxie as ordinate, length of muscle submitted to electric current as abscissa.

directly as we have always done in our previous experiments in a purely empirical way.

We can now see the very symmetrical correspondence between muscular stimulation and nervous stimulation; for nerve, the electrodes must be kept apart at a distance at least equal to a certain minimum, otherwise the chronaxie measurement is falsified by the reciprocal action of electrotonic polarizations. For muscle, the cathode must remain, as to its size, below a certain maximum dimension, otherwise the measure is falsified by an α effect. Though this effect had been left undetermined, our previous results [Lapicque, 1931 *b*] show again a great symmetry between the conditions for this effect on muscle and the conditions which

provoke an electrotonus on nerve; for nerve the current reaches the excitable part, the axone, through a less conducting sheath; for the α effect, the current reaches the excitable part, the muscle itself, through a sheath of Ringer more conducting than the muscle itself. We can conceive that these two opposite conditions, if combined, might more or less neutralize each other. This could explain how a nerve electrically stimulated through Ringer solution might present but a slight α effect [Lapicque, 1931 *b*, pp. 237 *et seq.*).

Now if we try to imagine what the α effect might be in itself, this comparison with the nerve inclines us toward the idea of a polarization, as I suggested in the preceding paper. This idea, unprecise when apparatus derived from Rushton's [1930] was used, becomes easy to determine if we consider tubular electrodes, that is to say, cylinders of Ringer surrounded by an insulating sheath.

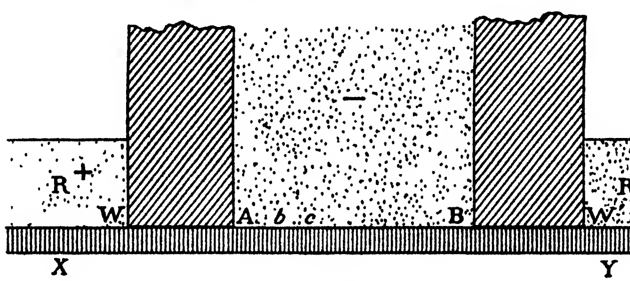

Fig. 3. Theoretical figure for the understanding of the retrograde polarization (see text).

Let us consider a muscle represented by a single fibre[1]; we shall use as a basis for our scale its crosswise dimensions. *AB* is the diameter of the Ringer solution which touches the fibre; *AW* and *BW'* the cross-sections of the wall on the same diameter; *R* and *R'* the Ringer solution outside the negative electrode. Suppose the anode is placed in this solution on the left of the figure (in *R*). When we create a potential difference in the circuit, the stimulating (*i.e.* negative) current must pass from *AB* to *R* through the muscle; of course, at first it will mostly pass in *A*, as in a vertical cylinder of Ringer the way to any point in *AB* offers the same resistance; but the next conductor constituted by the muscle offers a resistance proportional to its length, therefore weaker

[1] Concerning the muscle, the word fibre is not unequivocal; I mean Leydig's primary bundle, which I now call myon, that is to say the functional muscular element, just as a neuron is the functional nervous element [Lapicque, 1932].

from A than from any other point and becoming greater the farther we go from W. But the muscle surface is polarizable, which means that a counter-electromotive force is developed on each surface element, more or less rapidly, according to the intensity of the current going through this element. Thus A will be polarized most rapidly and then the current will be diverted toward b, where polarization will again increase and divert the current still farther. When the polarization thus reaches B, the current will be obliged at last to overcome directly the counter-electromotive force of polarization and to finish, in the muscle, the development of the process from which stimulation arises.

We see then that the minimal time during which we must keep the circuit closed for a given potential, in order to stimulate, is composed in the abstract of two distinct elements:

(1) the time necessary for polarization to develop itself along AB, time which of course is a function of this length;

(2) the time necessary to terminate the normal process of stimulation, which is a function of the intensity according to the properties of the tissue. This factor is normally given by the chronaxie.

With very small electrodes, the polarization difference between A and B can be practically neglected. We are dealing purely with the excitation time, and under those conditions the liminal duration for twice the rheobase is a true chronaxie.

When AB becomes longer, we have, for each intensity, first a polarization time, then an excitation time; the sum of these two time factors is, necessarily, for any intensity, greater than the pure and simple excitation time; this is particularly true when after the measure of the liminal intensity for long durations we look for the liminal duration with a double intensity. Whether the first intensity differs or not from the real rheobase, the duration obtained is different from the chronaxie; we readily see how right we are in calling it a pseudo-chronaxie; it is always greater and may become considerably greater if polarization takes a long time, and this happens when it has to cover a large surface. With an electrode of 2–3 mm., the pseudo-chronaxie is 2–3σ. We can conclude that here the polarization time already constitutes the greater part of this duration.

With Rushton's apparatus, where the whole muscle is dipped in Ringer, the current reaches it lengthwise through the solution; the physical reasoning is slightly different and we must take into consideration the difference of conductivity between the solution and the muscle.

But, in such a bath, the problem is not definite even if we localize the anode or the cathode in the bath by means of a metallic conductor as I did. I had supposed already [Lapicque, 1931 *b*, p. 242] that it was necessary to think of a polarization pushing back the current from spot to spot, but I did not know where to place the starting-point.

The following apparatus allows us to pass from one case to another and to determine the phenomenon which is common to both. Let us take a layer made of a given number of pieces of filter paper, 5 or 6 mm. wide and about 10 cm. long; let us soak it in Ringer and lay it horizontally between two vertical blades made of chlorinated silver; we then pass an electric current through it lengthwise. A carefully dissected sartorius, having been immersed in Ringer for more than half an hour, is placed flat and lengthwise on this pad. We can readily avoid the singular points constituted by the ends of the muscle in placing these on a thin glass slide (microscopic cover slide). The whole preparation is placed in a rectangular trough, made of parallel glass plates and covered with the same in order to avoid drying, without hindering observation of the muscle.

In this position we can easily stimulate the muscle with potential differences of a few volts; the curves obtained in this way are a mixture of α, γ, and even δ curves.

This is an example.

Exp. November 23. Sartorius of *R. fusca* placed as described above, with the cathode first on the distal side, *S*, then on the pelvic side, *P*.

Duration in σ	Liminal voltage *S*	Liminal voltage *P*
50	—	10·2
20	8·3	10·6
10	—	12·5
5	8·3	14·2
3	8·3	15·3
2	8·3	—
1	9·0	15·5
0·5	10·6	17·2
0·4	—	18·5
0·3	13	—
0·2	16	—

It is obvious that *S* is a γ curve; *P* starts in an α curve interrupted around 4σ by a γ curve. In other words we get exactly the same results as in a bath[1].

[1] This experiment is particularly similar to one published and illustrated with a graph in a preceding paper [Lapicque, 1931 *a*, p. 203].

But we can follow more clearly the nature of the response and particularly the change at the time when the curve is changed into a γ one, that is to say when the direct muscular stimulation becomes a stimulation through the nerve. Besides, we can observe the localization at the very beginning of the muscular response; we shall consider this point again further on. With this apparatus, the notion of parallelism without distortion of the lines of current, which was the original desideratum of Rushton's work, proves to be entirely delusive. This parallelism exists only in the pad, when considered alone, but it cannot subsist when the current goes through the muscle and stimulates it; one can see that it must deviate, and divide, at first according to the relative conductivity of the two superposed conductors, muscle and pad; then immediately after it must undergo some considerable distortion owing to the polarization of the muscular surface[1]. But here, no more than with the bath, there is no definite starting-point for polarization. The following procedure will give us one.

Let us cut into equal parts the pad soaked with Ringer and put them about 1 cm. apart; then fill this space with an insulating block (paraffin) of the same thickness. The muscle is then replaced on the pad as previously, but now it constitutes a bridge between two conducting parts and the whole current must pass through it. Let us place a sartorius in this position; its middle part on the paraffin, its extreme pelvic end on a slide and the part in between, 6 or 8 mm. of muscle, on the wet pad. The cathode being under the pelvic end, the curve obtained is a mixed α and γ curve.

Example. Exp. of January 4. 7 mm. between the slide and the paraffin.

Duration	Liminal voltage
100σ	8·2
50	8·8
40	9·6
30	10·6
20	11·9
10	12·7

The response of the muscle to this last stimulation is a strong contraction, even for the threshold current, which means that we have begun to stimulate the nerve; and if we continued from there toward

[1] I started with A. M. Monnier to study such distortions with the cathode ray oscillograph. On preliminary trials we found that the current going from a given point of the pad to one point of the muscle shows, in terms of time, a very complex shape, so complex that they could not be analysed in a rapid study.

short durations we could get a γ curve. The figures for longer durations belong conspicuously to an α curve which shows, by extrapolation, a pseudo-chronaxie of 8 or 10σ; this is the same length relation as with the other apparatus. We must be dealing with the same mechanism.

We can reason in the following way: the negative current will penetrate the muscle on the whole length *bg*, but the bulk of soaked filter paper, *F*, being more conducting than the superposed muscle, the intensity of the current will be the greatest in *b* and the smallest in *g*; so that *b* will be polarized quicker than any other point. This is the same case as the one described above, when the current arrives at a right angle in contact with the muscle, through the electrodes. But now the part played by the conductivity of the medium outside the muscle, as it had been observed in the bath, is obvious.

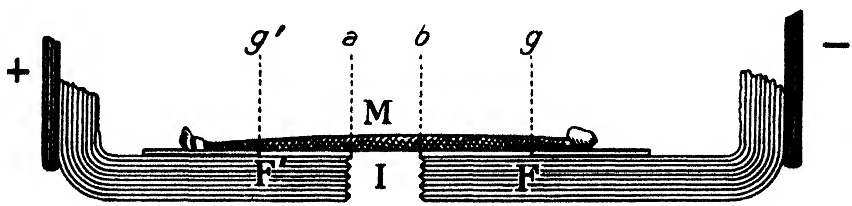

Fig. 4. Experiment upon an interrupted pad (see text).

When the retrograde polarization has covered the whole length *bg*, the current, as we have said, will be at last obliged to complete the stimulation process. But where will stimulation start? It would be rather hard to foretell it by *a priori* reasoning. If we did not take into consideration the interfacial polarization we should evidently think of it as occurring in *b*, at the end of the more conducting electrode, where the intensity of the current is the highest; but there the intensity will decrease gradually while it will increase in *g*; the question becomes very complex; and even if we knew the exact shape of these varying currents, it would still be difficult to foretell which one will reach the threshold first.

Observation answers very clearly: at least for the conditions of resistance of my experiments, stimulation starts in *g* and even 1 mm. back, upon the glass slide.

If we did not bear in mind the idea of a retrograde polarization, this result, I think, would seem paradoxical. This paradoxical character becomes still more striking if instead of the soaked paper we use a metallic electrode.

Let us consider a silver blade *pp'*, with the edge *p* well rounded; it is held by an insulating block *I*. We put, on this blade, the pelvic half of a sartorius, the hanging distal end of which is dipped in a Ringer bath *R*; a glass slide *g* insulates, as previously, the pelvic end. The silver blade constitutes the cathode, and the bath the anode. We get well-characterized α curves for durations around a hundredth of a second; for durations of thousandths of a second, where consequently the potential must be high to reach the threshold, the results are very irregular, and furthermore, they leave some permanent changes in excitability.

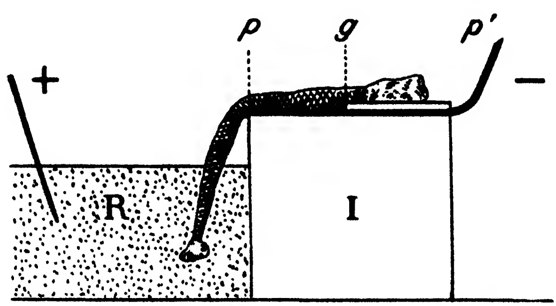

Fig. 5. Experiment upon a large metallic cathode (see text).

But the series of measurements made only with long durations give a very definite and perfectly significant strength-duration relation. They give a pseudo-chronaxie related to the muscle length lying on the electrode which is an exact agreement with the preceding results.

Example. Exp. January 7. 7 mm. between *p* and *g*. Cathode connected in *p'*.

Duration	Liminal voltage	
100σ	4·0	3·0
50	4·3	3·0
30	4·5	3·4
20	5·0	4·0
10	6·8	5·4

The two series of liminal potentials have been determined in the same order as given in the table; there were, between the two, a series of trials which did not give a consistent threshold with a duration of 5σ; these two series give very similar curves with a slightly extrapolated pseudo-chronaxie of about 8σ for each one.

When the direction of the current is reversed, we get a γ curve with a chronaxie of 0·4; the response is *d'emblée* large and characteristic of nervous stimulation. When *p'* is negative and the duration short, the

irregularity of response is accompanied by irregular thresholds; but in the series of determinations given in the table, that is to say purely in the range of the α effect, the first response, the real threshold, appears on the edge of the slide g; if we increase very gradually the intensity of the stimulus we can see the increase of the local response *pari passu*, and then for an intensity still slightly greater, it spreads all at once to the whole length of a few fibres.

In short, we get exactly the same phenomenon as with the preceding apparatus; and, what is still more remarkable, the same chronological characteristic for the same length of muscle, in spite of the difference of conductivity which is considerably greater; it is true that the blade is made of polished silver, which is thus polarizable and might have been the seat of a more important polarization than the muscle surface in contact with the Ringer; we should take into consideration the distribution of resistances in the different parts of the circuit. But these details, seeming not to have much physiological importance, are not worth much work; it is sufficient to note, with the metallic electrode as well as with the physiological solution: (1) the introduction of an α effect with a pseudo-chronaxie of about 1σ per mm. of muscle length undergoing the action of the current; (2) the origin of stimulation at the point of contact between the muscle and the electrode nearest to the origin of the stimulating current; that is to say the reverse of what a reasoning based only on ohmic resistances would give.

The case of the silver wire coming in contact with the muscle through the solution, and which at first approximation I considered as "stigmatic," is in fact a mixed case; the surface by which the electrode itself comes in touch with the muscle is evidently within the limits where the retrograde polarization is negligible; if the current reached the muscle only through this surface, we should get a correct chronaxie; but the bath, with its intermediate conductivity, diffuses above its end a portion of the current which is not negligible; thus our efficient electrode is wider than the wire; the value of the pseudo-chronaxie obtained with this procedure, $0{\cdot}7$–1σ, suggests an effective size of about 1 mm.

The same thing happens when the glass capillary electrode is not applied closely to the muscle surface; if its section makes a slight angle with it, or is slightly above, leaving a small space where the current can spread, the liminal duration for a current twice the rheobase appears increased, sometimes considerably. This can be easily understood and can be experimented upon directly; we have only to repeat the two measure-

ments (rheobase and chronaxie) at the same time as we change the height of the electrode by means of a micrometric screw; provided that the electrode obviously touches the muscle its pressure has no effect on chronaxie, but when it is raised above a certain level the smallest rise increases it notably, and it may become twice or three times larger. Evidently we are now dealing again with a pseudo-chronaxie similar to the one obtained with wide electrodes.

So that in order to have a correct measurement of a muscular chronaxie we must use either a metallic wire touching the muscle in the air[1], as we had commonly done until now, or a liquid electrode contained in an insulating sheath and whose diameter is less than $\frac{1}{2}$ mm.

Let us go back now to Keith Lucas's fluid electrodes. This is the way in which we can imagine these phenomena; the negative current penetrates first in the muscle at the level of the slot where it emerges from the Ringer, in the same way as at the end of the wet pad in contact with the paraffin; then a retrograde polarization starts and goes upward along the part of the muscle suspended above in the electrode; then, and then only, does stimulation take place; for the pure muscular stimulation this is true only when the slot is placed on the nerveless portion of the muscle.

[1] We proceeded in this way in most of our researches on the gastrocnemius; we considered the sartorius unsuitable on account of its variable increasing chronaxie and of its rapid loss of excitability. These reasons led Keith Lucas to devise his "fluid" electrodes in order to avoid desiccation and temperature changes which, he thought, were the causes of this variability.

But Dulière and Horton [1929] and Horton [1930] in a very elegant piece of research, whose practical importance seems to me very great, have shown that this muscle, and the others to a certain extent, are subject to a reversible loss of excitability, which takes place at ordinary temperature in a moist atmosphere without any desiccation and which returns when the muscle is thoroughly washed in Ringer; besides this alteration can be prevented if we previously wash the muscle in Ringer to take away a fraction of its potassium.

Hill, in his famous experiments on thermic phenomena of muscular contraction, had found it necessary, empirically, to keep the sartorius in a Ringer bath; Keith Lucas's "fluid" electrodes, though conceived with a different theoretical idea, met the same requirement; they ought to preserve the muscle excitability, but they introduced, in the way that we shall explain hereafter, the α effect; in which, at first, I thought I recognized the alterability mentioned above.

To-day, after Dulière's and Horton's work, we can use the sartorius without any hesitation for excitability researches. This muscle is particularly convenient for many physiological purposes on account of its anatomical conditions, and, as a matter of fact, it had been very often chosen for researches. It keeps perfectly in Ringer and, concerning chronaxie, gives correct values deprived of any α effect with capillary electrodes of an inside diameter of a few tenths of a millimetre.

This conception quantitatively accounts for the different results obtained by the authors: that is to say,

(1) The dimension of the slot has no influence whatsoever on the chronological characteristic of the intensity-duration relation. Indeed, Watts found no difference for two slots being in the ratio 1–3·9.

(2) The pseudo-chronaxie which measures this characteristic varies from 2 to 6σ. In our conception, this value must be a function of the length of the muscle undergoing the lengthwise action of the current; our experiments have found it to be approximately proportional to this length, about 1σ per millimetre. Here the length to be taken into account is the one of the muscle left above the slot. The authors did not indicate this length, as they did not suspect its interest. But both Keith Lucas and Watts, when taking care to place the slot on the nerveless portion of the sartorius, must have left only a few millimetres above the slot, and this agrees exactly with the values they obtained for the pseudo-chronaxie.

Summary.

Measurements of chronaxie have been made, on the pelvic part of the sartorius previously bathed in Ringer's fluid, with electrodes made of capillary glass tubes of varying diameters. Between 0·1 and 0·5 or 0·6 mm. the chronaxie remains fully invariable and equal to the one given by a silver wire taken as electrode on a muscle in air. The value so found, $0{\cdot}3\sigma$ as a rule, is the true chronaxie. From 0·5 to 0·6 mm. up, chronaxie, or better pseudo-chronaxie, increases regularly with the size of the cathode, reaching a value as high as 2 or 3σ for a diameter of 2 or 3 mm.

It is recalled that experiments made following Rushton, *i.e.* when a muscle is stimulated through a bath of Ringer's fluid, give pseudo-chronaxies regularly varying with the length exposed to the current, viz. 2 or 3σ for 2 or 3 mm., and about 1σ more for each additional millimetre. Thus, both series of experiments join exactly.

Reasoning physically from the case of large capillary electrodes, one must admit a polarization of the muscle surface under the cathode, starting from the edge at the point nearest to the anode, and developing itself progressively as far as the most remote point of the cathode. So, the current crossing the muscle surface is diverted backward all along the diameter of the cathode. In order to reach the threshold, the circuit must remain closed a sufficient time to allow (1) this polarization to come to its end; (2) the proper excitation process to be achieved. This sum

appears in experiments as pseudo-chronaxie, evidently longer than the true chronaxie, and increasing *pari passu* with the length of muscle subject to the phenomenon.

Laying the muscle upon a pad soaked with Ringer's fluid and sending an electric current in parallel lines, we find the same α and γ curves as in a full bath. This still holds when we interrupt the pad with a block of insulating substance and bridge this electric gap with the muscle. Such a case appeals to the same physical reasoning as the case of a large capillary electrode, but involves as a necessary condition a greater conduction in the electrode than in the muscle.

Every condition and every phenomenon related to this retrograde polarization for the muscle is symmetrical to the physical electrotonus in the nerve, where, as classically and rightly admitted, the excitable substance (axon) is surrounded by a less conducting substance.

Post-scriptum versus W. A. H. Rushton.

Following the report [Rushton, 1930] which I discussed in my preceding papers [Lapicque, 1931 *a*, *b*] and am still discussing in the present one, Rushton has published several papers on the same subject, bringing forward, with much application and ingenuity, new and detailed evidence concerning the fundamental phenomenon which is the basis of the discussion, viz. the existence of two different strength-duration curves for the nerve-muscle complex when stimulated electrically through Ringer's solution. I have said already, and willingly repeat, how much I am indebted to Rushton for having made this fact unquestionable even in normal fresh muscle, and so for affording the opportunity of evaluating the rôle played by the electrolytic medium. But, I must confess, I can hardly find in all this secondary work any new light on the physiological problem concerning the interpretation of the α curve and consequently the question whether the neuromuscular isochronism is right or wrong.

In a first paper [Rushton, 1931] he shows that the phenomenon is present, generally speaking, in any one among the frog's striated muscles. Since I discarded, the moment I read his primary report [Rushton, 1930], my old tentative explanation of the Lucas's α curve, I never regarded such a generalization as in doubt and I even admitted it implicitly, considering at every theoretical standpoint, the *muscle*, or the *muscular substance*, without any restriction to a particular case. Though I have borne in mind a special condition for the Gastrocnemius, the customary object of my previous experiments; here the muscular fibres, admittedly able (theoretically) to give an α effect as well as those of any muscle, are geometrically ordered according to the well-known penniform structure of the whole muscle; thus they present only a small part of their length freely to the current flowing in the Ringer; such a condition ought to restrict the phenomenon. In fact, in this muscle, Rushton found but a very small difference between the two curves, so small that plotted in the same way as other muscles, it was not conspicuous at all, and appeared only with special contrivances [Rushton, 1931, p. 270]. [Incidentally, one may note that the change in latent period showed by Rushton [1931, p. 275], when stimulation passes from γ to α, agrees fully with my theory stated above, viz. retrograde polarization.]

In a second paper [Rushton, 1932 *a*] he shows that the muscular fibres giving the α curve are those responding to the nervous stimulation. I do not know that anybody

has ever ventured the contrary hypothesis, surely a very strange one. For my own part, I did not raise any question about this connection, in the whole course of the discussion I gave in two reports published in this *Journal*, 5 or 6 months before Rushton's quoted paper was printed, but, unfortunately, after it was written, as we learn from a footnote. I understand that Rushton would not throw in the waste-paper basket a very industrious work, in any case interesting from its technical ingenuity, but at the time of publication he could certainly have left out such a cutting conclusion as the following: "The α fibres being supplied by nerves and having a chronaxie of the order of hundred times as long as nerve...the conclusion leads inevitably to the rejection either of Lapicque's theory of isochronism or of the block electrode method of obtaining the chronaxie."

I quite agree to the position of the dilemma, but the question of the legitimacy of the said block method was precisely raised by myself half a year before, with a sufficient emphasis, I supposed; I had given a series of arguments against this legitimacy, and I was anxious to know what criticism would meet these arguments; but Rushton's paper remains systematically ignorant of them, and, furthermore, assumes without reserve as well as without justification that it was dealing with an alpha substance or even, as we have seen in the sentence quoted above, alpha fibres, a new and aggravating expression, though, in the preceding paper [Rushton, 1931, p. 265], a footnote admitted that two modes of excitability are not a sufficient evidence for the existence of two substances.

In a third paper [Rushton, 1932 *b*] we get the discussion, but not yet in the depth of the problem, only on a theoretical consideration raised by myself merely as preliminary. We must now satisfy ourselves with this unimportant discussion, for the fourth paper [Rushton, 1932 *c*], the last at the moment, is devoted to the demonstration that the γ curve, as a rule in a Ringer's bath, when the field of action to the electric current is large, is related to the nerve; again a good piece of ingenuity and precision, but quite superfluous, the fact being not denied.

The fundamental question is to know if the slow chronological character of an α curve means a large chronaxie for the muscle on which it has been obtained. I said no, and I say no.

Rushton seems to believe that my criticism aims only at the wording, and in deference to my rights as author of the name chronaxie, he concedes this name "to be used in the sense which Lapicque wishes"..."in the case where the strength-duration curve is canonical or where it satisfies any new criteria which Lapicque may in future suggest" [Rushton, 1932 *b*, p. 439].

I am certainly not inimical against an ironical joke in a scientific discussion, but I think it cannot be substituted for real arguments. This canon, that Rushton endeavours to turn to ridicule, has been imagined with the aim of compelling Rushton himself to acknowledge a notable difference between the α curves and the time relation having given birth to the chronaxie; if so, the duration corresponding to twice the liminal strength for long durations, in an α curve, should evidently not be homologous with the chronaxie and could be named a false chronaxie. Rushton considers this reasoning as an arbitrary fancy of my own; furthermore, he lays stress on this fact, long ago recognized by myself, that with the very rapid nerves and for durations shorter than the chronaxie, the strength-duration curve diverges from the general law. I had formerly imputed this deviation (we are dealing with durations of a tenth of a sigma, and below) to the self-inductance in the stimulating circuit, maybe wrongly (still I was not guilty of a certain technical fault that Rushton attributes to my experiments in contradiction to the very text of my report); that is a question concerning the physiological theory of the stimulation, but neither the measurement of the chronaxie, nor the said canon, since the alleged error lies outside the part of the curve used in the computation. I have twice asked Rushton by letter to let me know the figures he obtained between rheobase and chronaxie, the only

figures bearing on the case; he did not answer; thus I suppose his figures have been canonical, as well as those of any author, except perhaps very close to the chronaxie itself, where there may already exist a deviation of but a few units p.c., *i.e.* of the order of the experimental approximation. That is by no means comparable with the deviation offered by the α curves, chiefly on the side of long durations, and giving frequently incertitudes of a 100 p.c. in the position of their chronaxie, or better pseudo-chronaxie [Lapicque, 1931 *a*].

But whatever the criticism against it, the canon itself matters now very little. Its value was only previous and temporary, indicating that another quantitative relation could mean another quality of phenomenon. I have tried to find this other phenomenon, and in due course, I hope I have succeeded in tracing it. "Until the laws governing the wide variations (according to the size of electrodes) can be worked out, it does not seem possible to speak of any single chronaxie as characteristic of a given muscle" said Davis nine years ago, in a sentence quoted in the above paper and recalled by Rushton. Perhaps, the above report gives such laws, and shows when and how a neatly defined chronaxie exists for a given muscle, being equal to the chronaxie of the corresponding nerve, and when and how a parasitic phenomenon falsifies the strength-duration relation, leading to senseless chronological values. The time constant of an α curve, theoretically suspected at first because uncanonical, is now experimentally convicted as a false chronaxie, without any physiological meaning.

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STUDIES CONCERNING THE ALIMENTARY ABSORPTION OF WATER AND TISSUE HYDRATION IN RELATION TO DIURESIS.

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Part III. The influence of posterior pituitary hormone on the absorption and distribution of water.

Introduction.

It is known that the diuresis which normally follows a large dose of water is inhibited by pituitrin and likewise the polyuria of diabetes insipidus is usually reduced. It was shown by Verney [1926] that the abundant hypotonic urine formed by isolated kidneys when perfused by a simple heart-lung preparation is both reduced in amount and increased in concentration by pituitrin or by the inclusion in the blood circuit of a head, provided the pituitary gland of that head remains intact. Although the polyuria of isolated kidneys is not a water diuresis, since no additional water has been given, the inhibition of their activity by pituitrin is an indication that the hormone of the pituitary body has a direct renal action and suggests [Verney, 1929] that this inhibition of water diuresis is primarily of renal origin. This is further supported by the observation of the same author that in unanæsthetized dogs with denervation of one kidney the urine flow from the ureters is normally equal and the kidneys respond equally to water diuresis and are equally inhibited by pituitrin. On the other hand, except by some extrarenal action, it is difficult to explain the results of Miura [1925], and Buschke [1928], who observe increases in the blood chlorides when pituitrin is given to nephrectomized rabbits.

Our object is to examine the effect of this hormone upon the absorption and distribution of water administered by stomach tube to intact unanæsthetized animals.

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Firstly, we have administered water to rats and compared the normal absorption curve with the absorption curve after pituitary extracts by killing sets of rats at varying times after water was given and determining the amounts of water remaining in the alimentary canals. Secondly, in rabbits we have obtained for analysis samples of muscle, skin and blood during the course of the experiments by the procedures which have been shown in the first paper of this series to leave the diuresis uninfluenced and afterwards we have made post-mortem examinations.

A. The absorption of water from the alimentary canal of rats under the influence of pituitary hormone.

Experimental procedure.

The rates of water absorption of rats which have received about 1 unit of pitressin per 100 g. rat subcutaneously 30 min. before the administration of 5 p.c. of their body weight of warm water have been compared with the absorption rates of rats receiving only the dose of water. The method of study is precisely as described in the first paper of this series, Part I, Section B.

Results.

Table I gives the weight of gut and contents at varying times after administering 5 p.c. of the body weight of water. The left-hand columns are control experiments, the right-hand columns give the results on animals under the influence of pituitrin. It will be evident from Table I and Fig. 1 that the pituitary hormone has no significant influence on the water absorption rate in rats.

Table I.

Time after giving water (min.)	Rats without pitressin			Rats with pitressin		
	No. of rats	Weight of gut as p.c. of body weight	Average	No. of rats	Weight of gut as p.c. of body weight	Average
15	3	8·9, 8·5, 9·2	8·9	4	10·4, 8·3, 7·1, 8·9	8·7
30	4	9·6, 6·5, 4·8, 6·9	7·0	4	7·2, 6·7, 6·4, 6·8	6·8
45	6	6·9, 6·2, 6·4, 6·1, 6·2, 5·7	6·2	6	5·2, 7·1, 6·2, 7·6, 6·6, 6·4	6·7
75	7	4·6, 10·3, 6·4, 7·9, 5·9, 5·5, 4·9	6·5	7	4·8, 8·4, 6·6, 8·9, 10·9, 4·7, 5·8	6·9
120	6	6·8, 6·2, 7·4, 7·6, 5·9, 4·8	6·5	6	5·2, 6·8, 6·8, 6·3, 5·9, 6·6	6·3
Controls, no water	5	6·6, 6·2, 8·6, 5·6, 5·4	6·5			

Discussion.

In rats water given per os is absorbed as usual under the influence of pitressin, but is only excreted at a greatly diminished rate by the kidney. This water, which forms 5 p.c. of the body weight of the animal, in a period of about ½ hour is absorbed and either stored in the blood and

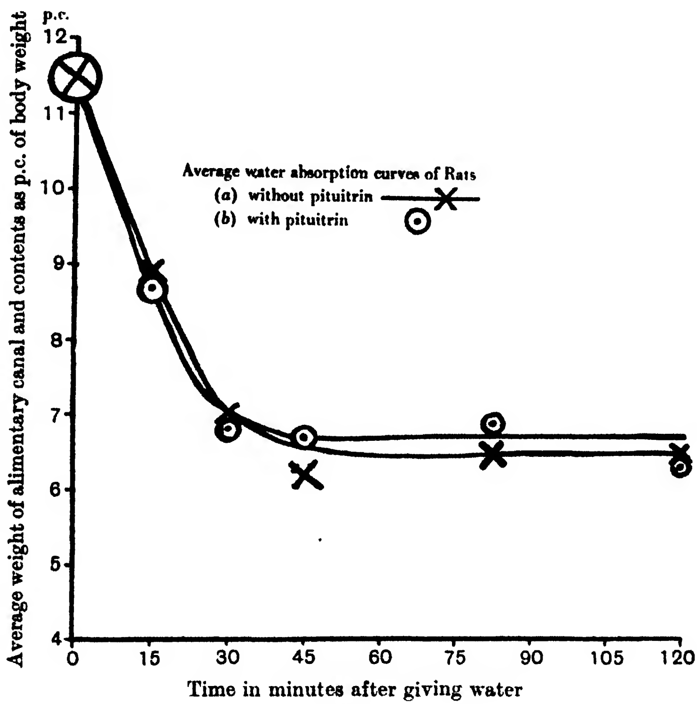

Fig. 1.

tissues of the animal or excreted by some channel other than the kidney. The only channel not under direct observation is the respiratory tract. As a precaution, therefore, a number of animals were weighed some hours after giving the water to make sure that such water as had not been excreted by the kidneys was still present in the body. There was indeed a loss of weight, but this did not account for the greater part of the water which was evidently stored in the blood and tissues of the animal.

Since it is difficult to take tissue and blood samples from a small animal such as the rat it was decided to continue our investigations on water distribution in another animal, the rabbit.

B. The storage of water in the muscle, skin, blood and serous cavities of rabbits during the inhibition of a water diuresis by pituitary hormone.

Experimental procedure.

0·2–0·4 c.c. of pitressin was given 30–45 min. before water administration. The animals then received 4 p.c. of their body weight of water by stomach tube and samples of muscle and blood were taken by the procedure outlined in the first paper of this series [Heller and Smirk, 1932]. At the conclusion of the experiment the fluid contained in the serous cavities was collected and measured.

Results.

When diuresis was inhibited by pituitrin the water content of the blood was increased to a much greater degree than it was in cases where the inhibition was produced by anæsthesia (see Part IV) or laparotomy (unpublished observation). Also the water uptake of muscle was greater where diuresis was inhibited by pituitrin than where diuresis was allowed to proceed normally. These results are summarized in Table II.

In two very nervous rabbits, Nos. 6 and 7, this increase in water content of muscle was slight, which suggested that perhaps there was an inhibition of alimentary absorption as well as of renal activity.

In post-mortem examinations made at the end of the experiments, but unfortunately only on the last six animals, quite large quantities of fluid were obtained from the peritoneal cavities: 15, 20, 20, 10, 4 and 14 c.c. respectively. Not only was there more fluid in the peritoneum but also there was more in the pleural and pericardial cavities than had been found in six of the rabbits where no pituitrin had been given and an examination of these cavities made at the conclusion of the experiment. In these latter animals there had been no inhibition of diuresis.

It is clear then when water is absorbed from the alimentary tract and fails to be excreted by the kidneys there is a greater increase in the water content of muscle and of serous cavities. Our methods, therefore, detect this water when it is absorbed, and our previous and subsequent observations concerning the absence of an appreciable increase in the water content of muscle must be regarded as strong evidence that when diuresis has been inhibited without an appreciable increase in the water

TABLE II.

Animal No.	3-hour output of urine: Normal c.c.	3-hour output of urine: Under pituitrin c.c.	Increase in p.c. water of muscle	Degree of blood dilution (as a percentage of the initial concentration)	Post-mortem examination after 3 hours	Notes
1	57	1	+3·0	—	None made	Previous diet of oats, 0·2 c.c. pitressin
2	42	11	+3·2	—		
3	48	10	+4·4	—		
4	84	18	+2·7	—		
5	76	57	+2·5	—		
6	—	10	+0·5	93·5		Previous diet of cabbage, 0·3 c.c. infundin
7	—	30	+0·4	94		
8	89	11	+1·2	91	15 c.c. of fluid in serous cavities	Cabbage diet, 0·5 c.c. infundin
9	98	22	+1·8	88	20 c.c. of fluid in serous cavities	—
10	92	10	+2·0	95	20 c.c. of fluid in serous cavities	Cabbage diet, 0·2 c.c. pitressin
11	66	9	+1·9	92	10 c.c. of fluid in serous cavities	Cabbage diet, 0·2 c.c. pitressin P.D.
12	49*	4	−0·2	94	4 c.c. of fluid in serous cavities	Cabbage diet, 0·4 c.c. pitressin P.D.
13	67*	11	+1·3	88	14 c.c. of fluid in serous cavities	Cabbage diet, 0·4 c.c. pitressin P.D.

* These two animals passed copious fluid stools shortly after receiving their doses of water.

content of muscle there has been a reduction in the alimentary absorption of water (see Parts I and IV).

It is clear from Table II that an increase is obtained in the water content of longissimus dorsi muscle, but the degree of increase varies. The water uptake of a second muscle—the vastus externus—was next studied (Table III), to obtain an idea of how far the changes in the water content of one muscle were an index of changes in the musculature as a whole.

TABLE III. The uptake of water by two different muscles under the influence of pitressin.

Exp. No.	Increase in p.c. water of two muscles 90 min. after giving water: Longissimus dorsi	Vastus externus
1	+2·9	+1·9
2	+2·0	+1·6
3	+1·4	+1·4
4	+1·3	+1·6
5	+3·1	+2·0
Average	+2·1	+1·7

As a control observation the influence of pitressin alone on the water content of muscle was investigated in five animals. Beyond the fact that no additional water was given the experimental conditions were unaltered.

The changes in the water content of muscle were + 0·6, + 1·4, + 0·3, – 0·8, – 0·2, averaging + 0·3 p.c.

The corresponding control in which water but no pituitrin was given has been described in the first paper of this series.

Because the removal of as little as 2 c.c. of blood may be associated with a blood dilution in the subsequent sample [Smirk, 1932] and also because the loss of blood from muscle sampling though slight is indefinite, it has been thought desirable to make a few separate experiments on the blood dilution in animals where no muscle samples were taken.

The results are divided into two groups (Tables IV and V) which differ in the lengths of the time periods between giving pitressin and water and between giving water and taking the second blood sample. To the first group (Table IV), in which a considerable blood dilution is

TABLE IV. Animals received 0·2 c.c. pitressin ½–¾ hour before giving water and the second blood sample was taken 1½ hours after giving 4 p.c. body weight of water.

Exp. No.	Degree of blood dilution expressed as a percentage of the initial concentration: By hæmatocrit	By hæmoglobin	Urinary output in 1½ hours after water c.c.
1	93	86	0·0
2	—	96·5	1·0
3	98·5	96·6	5·0
4	92·2	87·8	4·5

TABLE V. Animals received 0·2 c.c. pitressin at least 2 hours before giving water and the second blood sample was taken 2 hours after giving 4 p.c. body weight of water.

Exp. No.	Degree of blood dilution expressed as a percentage of the initial concentration: By hæmatocrit	By hæmoglobin	Urinary output in 2 hours after water c.c.
1	98·8	101	0·0
2	101·4	105·4	4·0
3	100	99·0	0·0
4	107·3	100·4	5·5
5	110	104·8	6·0

the rule, may be added the results of eight experiments from Table II, in which the experimental conditions were similar. In the second group, of five experiments in all, there is no regular diminution in either the hæmatocrit reading or the hæmoglobin percentage. The only essential difference appeared to be that pitressin was given a longer time before the water in the experiments where there was no regular blood dilution.

For the present we do not intend to investigate this time difference in the action of pitressin or to confirm whether it is indeed a regular phenomenon. It may indicate some mechanism by which the partition of water between blood and tissues can be altered. There did not appear to be any defect in alimentary absorption.

Magnus and his school have investigated the part played by the skin in the storage of water and salt. Since water is normally stored in skin [see also Heller and Smirk, 1932] we decided to investigate the changes in water content after giving pituitrin and water.

A small piece of skin was excised from the back with the aid of local subcutaneous anæsthesia. The five animals were given 0·2 c.c. of pituitrin and then 4 p.c. body weight of water by stomach tube. After 90 min. a second skin sample was removed in the same way and the animals were killed. This procedure was repeated in a series of another five animals in which water but no pituitrin was given. At 105 min. after giving pituitrin and 90 min. after giving water the changes in the water content of muscle were: $-2{\cdot}5$, $-1{\cdot}0$, $-0{\cdot}8$, $-0{\cdot}3$, $-1{\cdot}2$, average $-1{\cdot}2$ p.c. In the control animals which received water only the changes were: $+3{\cdot}5$, $+1{\cdot}1$, $-1{\cdot}6$, $+0{\cdot}6$, $+5{\cdot}5$, average $+1{\cdot}8$ p.c. It appears that the water content of skin is diminished in the animals which received pituitrin injections despite the administration of additional water.

The influence of pituitrin upon the extrarenal water loss. In view of the marked variations in the extrarenal loss of water which followed changes in the temperature of the surrounding air (Part II) we considered it worth while to determine whether there was any appreciable change in the extrarenal water elimination when a water diuresis is inhibited by pituitrin.

In five rabbits the non-fæcal extrarenal loss of water was determined by weighing the animals before and 4 hours after a dose of water, and then again before and 4 hours after a dose of water together with 1 unit of pituitrin subcutaneously. The room temperature was $17 \pm 1°$ C. on both occasions.

The losses of water were 13·5, 16, 10, 8 and 10·5 g. in 4 hours, average 11·6, when the animals received water only, and 27·5, 16, 15, 31·5, and 30·5 g. in 4 hours, average 24·1, when the diuresis was inhibited by pituitrin.

Presumably, since rabbits do not sweat, this increased loss of water takes place through the lungs. The increased loss may be caused either by an increase in the moistness of the respiratory passages or by an increase in the pulmonary ventilation.

It is clear, however, that although the extrarenal water elimination appears to be greater after pituitrin yet the difference in the quantities of water lost is in no case sufficient to account for the inhibition of renal activity. More detailed confirmation of this point would not, however, be relevant to our present work.

Discussion.

It will be seen from the results obtained in rabbits (see Tables II and IV) that when pituitrin is injected subcutaneously ½ hour before the administration of a large dose of water so that the diuresis which normally results is prevented, there is usually a definite hydræmia. The water content of the muscles is also increased (Tables II and III), and the degree of this is much greater than that met when diuresis is allowed to proceed normally. In addition in all those cases where an examination has been made there was an increase in the fluid content of the serous cavities, so that whereas normally it is difficult to collect more than 1 or 2 c.c. of fluid, one is now able to obtain as much as 15 c.c. or 20 c.c. As a parallel observation one of us [Heller, 1930] has previously reported the distension of the lymph spaces in frogs. It was suggested by Rees [1920] and by Koref and Mauntner [1926] that failure of alimentary absorption might account for a part of the inhibitory action of pituitrin. In the rabbit it is clear, however, that there is a definite excess of water present in both musculature and blood. We suggest that, since inhibition is present when there is an excess of absorbed water to be disposed of, it is not likely that changes in the water absorption rate play any considerable part in the pituitrin inhibition.

It has been argued that the excess of water which has been shown to be present in the blood may not be free but in some physico-chemical way "bound." This, however, is unlikely, since there is regularly an increased fluid content of the serous cavities which would appear to result from the increased transudation following a dilution of blood colloids.

An increased "avidity" of plasma for water would hardly be associated with increased transudation from the capillaries into the body cavities, and any increased "avidity" of tissues would surely need to prevent blood dilution before it could be cited as a direct cause of diminished renal activity. Heller's work on frogs [1930] yielded no evidence of a direct action of pituitrin on the water metabolism of living muscle, or upon the physico-chemical properties of tissue proteins. Heller was able to show that the increase of weight in frogs under the influence of pituitrin is not decreased by a rise in body temperature.

It appears likely therefore that the mechanism of the inhibition of water diuresis by pituitary hormone is renal and not extrarenal. There remain facts which would be difficult to explain if it were assumed that all the changes in water metabolism after pituitrin were the result of the inhibition of renal activity.

From Table III it is clear that the water distribution and storage is not uniform throughout the musculature, and similar differences in the water uptake of different muscles have been reported by Baer [1926] and Tashiro [1926]. The results obtained on skin—in which the water content was actually reduced after pituitrin and water administration—make it clear that a complete picture of the water distribution could only be obtained by the analysis of several samples of every tissue and by the elimination of errors due to changing vascularity.

The fall in the water content of skin may very well be due to its lessened blood content, the percentage water content of blood being greater than that of muscle. If corrections could be introduced which eliminated this factor, it is possible that the water content of the actual skin substance would be increased. The actual amounts of water involved are in any case small since the skin forms only 12 p.c. of the body weight.

Poulsson [1930] concludes that the reduced output of water after pituitrin is due to an increased reabsorption of water in the tubules, since the action of pituitrin in inhibiting diuresis is not accompanied by any appreciable change in the calculated amount of glomerular filtrate.

His calculations of the amounts of glomerular filtrate require, however, the assumption that all the creatinine and sulphate excreted in the urine is filtered by the glomeruli and reaches its urinary concentration by water absorption in the tubules.

The internal localization of function in the kidney is a subject of great difficulty, and the work of Starling and Verney [1925] is not in agreement with the assumption which Poulsson and others have used to calculate the amount of glomerular filtrate.

SUMMARY.

1. In rats the alimentary absorption rate for a 5 p.c. body weight dose of water is uninfluenced by giving 1 unit of pitressin per 100 g. rat subcutaneously. This dose of pitressin considerably reduces the formation of urine.

2. In rabbits receiving 0·2–0·4 c.c. of pitressin and 4 p.c. body weight of water, there is reduced urine formation and increased water storage in the blood, muscle and serous cavities.

3. The nature of the anti-diuretic action of pituitary hormone is discussed in the light of these observations.

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Part IV. The influence of anæsthetics and hypnotics on the absorption and excretion of water.

Introduction.

It has been demonstrated by many workers, Schroeder [1888], Frey [1907], Molitor and Pick [1925, 1926, 1927], Smith and McClosky [1925], Stehle and Bourne [1928], Buschke [1928], Fee [1928], Kugel [1929], Bonsmann [1930], that the excretion by the kidneys of a large dose of water administered by stomach tube is rarely normal in an anæsthetized animal. The effect of anæsthesia appears to depend upon the nature of the anæsthetic, upon the depth of anæsthesia and also upon

the animal used. With most anæsthetics the urinary output is diminished, with a few it is increased. The school of Pick conclude that in general the influence of anæsthetics results from their action upon the central nervous system. Narcotics which depress first the higher centres such as paraldehyde, chloralose, avertin, and alcohol tend to increase the outflow of urine; those which first depress the mid-brain areas tend to diminish the activity of the kidney (luminal, chloretone). It is supposed by them that the normal control of diuresis is through a diuretic centre in the mid-brain: the depression of its activity diminishes renal activity; the depression of higher control centres with release of its activity is said to increase urinary outflow.

Though opinions may differ as to the precise way in which anæsthesia influences the output of urine there is little doubt that a number of substances of differing chemical structures but having in common the property of producing narcosis have also in common the property of reducing the urinary outflow. The object of this paper is to determine whether some alteration in the kidney or tissues is responsible for this anti-diuretic action or if alterations in alimentary absorption play a part.

In a few experiments performed by us on rabbits anæsthetized with ether it was observed that a dose of about 4 p.c. of the animal's body weight of water produced little or no increase in the output of urine. But samples of muscle in these animals showed only slight increases in their water content. Now in rabbits where renal activity was prevented by pituitrin but where there was every evidence of water absorption the extra water given was readily detected in muscle samples (see Part III). Therefore, the absence of any considerable increase in the water content of muscle in these anæsthetized rabbits suggested to us that a part at least of this apparent inhibition of renal activity might be of alimentary origin and due to the non-absorption of the water given.

For this reason we decided to investigate the matter more accurately, using the statistical method of studying alimentary absorption in rats already described in Part I of this series [Heller and Smirk, 1932]. The rat is particularly suitable for this investigation since we have shown that water absorption is practically complete before the onset of diuresis. It is, therefore, possible to investigate also the effect of anæsthesia on diuresis as distinct from absorption by administering the anæsthetic at a time when water absorption is almost complete and diuresis is only just beginning.

Experimental procedure.

Rabbits weighing about 2 kg. were used and a uniform dose of 75 c.c. of water was given. The methods employed were light ether anæsthesia, deep chloretone anæsthesia and light chloretone anæsthesia. The water and chloretone were given by stomach tube, the ether by inhalation.

The procedure for rats has been exactly as described in Part I, chloroform and ether were given by inhalation; paraldehyde, luminal, urethane and chloralose were given subcutaneously. The doses and previous diet are stated with the protocols. Body temperatures were measured per rectum.

Results.

Preliminary experiments on rabbits. Four animals received ether. Under ether their urinary outputs for periods of 4, 4, 3 and 4 hours were 2, 19, 5 and 0·5 c.c. as compared with their normal outputs of 29, 35, 36 and 52 c.c. for corresponding time periods. The increases observed in the percentage water content of muscle 1½ hours after giving water were only + 0·8, + 0·9, 0·0, + 0·8, averaging + 0·6 as against an average increase of + 2·0 p.c. where diuresis is inhibited to a corresponding degree by pituitrin (see Part III).

Four experiments, two with light chloretone anæsthesia and two with deep chloretone anæsthesia, gave a similar absence of appreciable change in the water content of muscle and no increase in the water content of liver (determinations made on 16 samples of muscle and 18 samples of liver). The technique of removing liver samples and the influence of the procedure of laparotomy upon absorption and diuresis will be described in a subsequent paper. The urinary output was also inappreciable.

Although the chloretone experiments are complicated by the intestinal method of administration as well as by laparotomy, the absence of expected increases in the water content of the tissues examined appears to be a justification for further work.

I. *The influence of anæsthetics on water absorption in rats.* It will be clear from the following results that the rate of absorption of water from the alimentary canal of rats is greatly diminished by ether (Table I)

TABLE I. Ether. Light ether anæsthesia with usually a positive corneal reflex. Deprived of water overnight.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
60 min.	5	9·0, 10·2, 9·5, 7·3, 8·7	9·0
Controls, no extra water	5	5·3, 5·8, 6·7, 6·8, 7·0	6·3

and chloroform (Table II); slightly diminished by luminal (Table III), paraldehyde (Table IV) and urethane (Table V), and hardly diminished by chloralose (Table VI). The inhibition of absorption produced by ether is unaltered by physostigmine (Table VII).

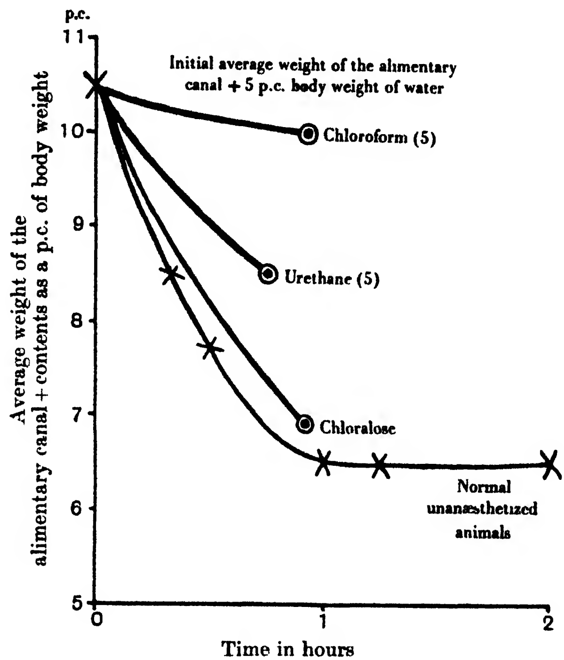

Fig. 2. The influence of chloroform, urethane and chloralose upon the alimentary absorption of water. (The animals were allowed access to water overnight.)

TABLE II. Chloroform. Light chloroform anæsthesia with usually a positive corneal reflex. Allowed water up to the time of the experiment.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
55 min.	5	10·2, 8·3, 10·7, 10·4, 10·4	10·0

TABLE III. Luminal. (0·015 g. of sodium luminal per 100 g. rat deprived of water overnight.)

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
30 min.	5	8·5, 8·8, 8·3, 11·5, 9·9	9·4
45 ,,	5	7·2, 7·7, 5·2, 9·5, 9·6	7·8
90 ,,	6	5·0, 5·8, 5·9, 7·6, 8·4, 6·8	6·6
Controls, no extra water	5	5·3, 5·8, 6·7, 6·8, 7·0	6·3

TABLE IV. Paraldehyde. (0·2 g. of paraldehyde subcutaneously per 100 g. rat. Deprived of water overnight.)

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
30 min.	4	8·4, 6·4, 7·2, 7·5	7·4
45 ,,	5	8·9, 7·5, 7·3, 7·4, 7·0	—
		5·2 (very lightly narcotized)	7·6
Controls, no extra water	3	6·3, 5·0, 5·1	—

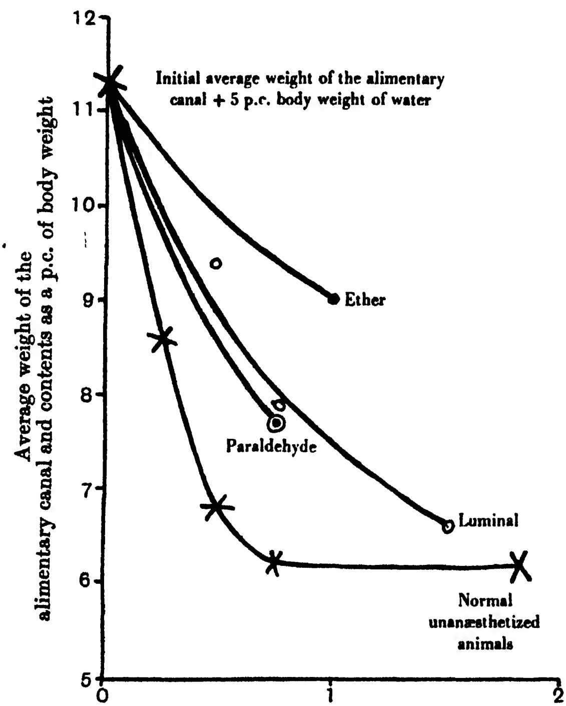

Fig. 3. The influence of ether, paraldehyde and luminal upon the alimentary absorption of water. (The animals were previously deprived of water overnight.)

TABLE V. Urethane. 0·12 g. of urethane per 100 g. rat. Water allowed up to the time of the experiment.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
45 min.	5	8·2, 7·4, 7·1, 8·9, 10·8	8·5
Controls, no extra water	7	6·3, 5·0, 5·1, 5·5, 6·8, 6·6, 5·5	5·7

TABLE VI. Chloralose. Two animals had 0·01 and one animal 0·008 g. chloralose per 100 g. rat. Water allowed up to the time of the experiment. Because of the relative insolubility of chloralose larger quantities of fluid had to be injected. The controls were given the same amount of 0·2 p.c. NaCl (2 c.c.) subcutaneously.

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
55 min.	5	6·0, 6·8, 8·9, 6·5, 7·0	6·9
Controls, no extra water	7	6·3, 5·0, 5·1, 5·5, 6·8, 6·0, 5·5	5·7

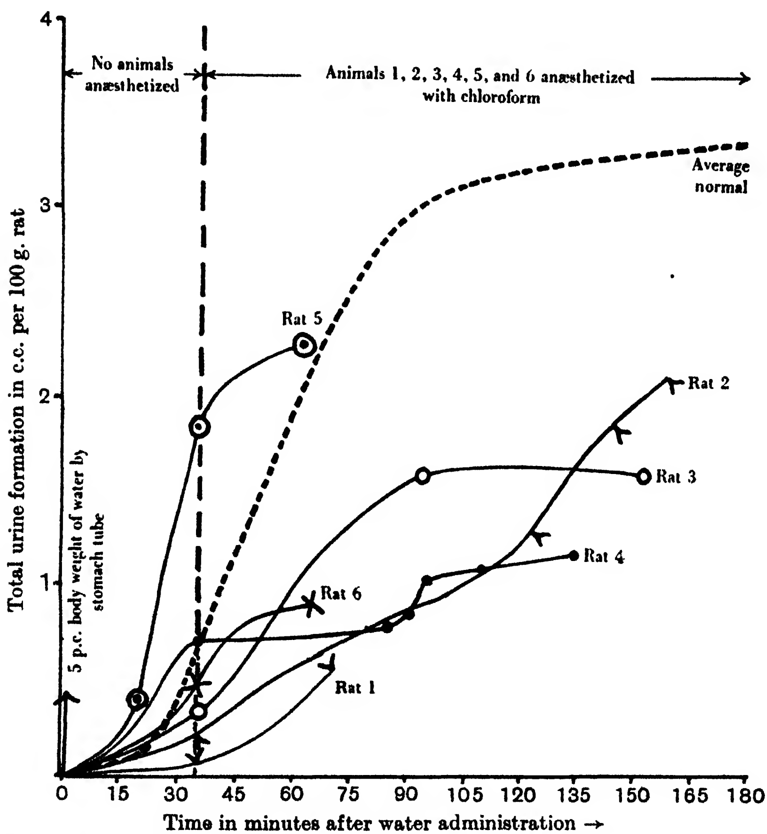

Fig. 4. The influence of chloroform anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

TABLE VII. Ether and physostigmine. Light ether anæsthesia with usually a positive corneal reflex. Deprived of water overnight. (Record of the dose of physostigmine lost.)

Time after giving 5 p.c. body weight of water	No. of rats used	Weight of the alimentary canal and contents expressed as a percentage of body weight	Average
60 min.	5	9·6, 9·1, 9·3, 8·0, 8·0	8·8

The results are summarized in graphical form on Figs. 2 and 3, on which the normal absorption curves described in Part I are reproduced. Since it is not possible with injected drugs to obtain much uniformity in the degree of narcosis except by repeated trials on the same animal an increase in the variability of results is to be expected.

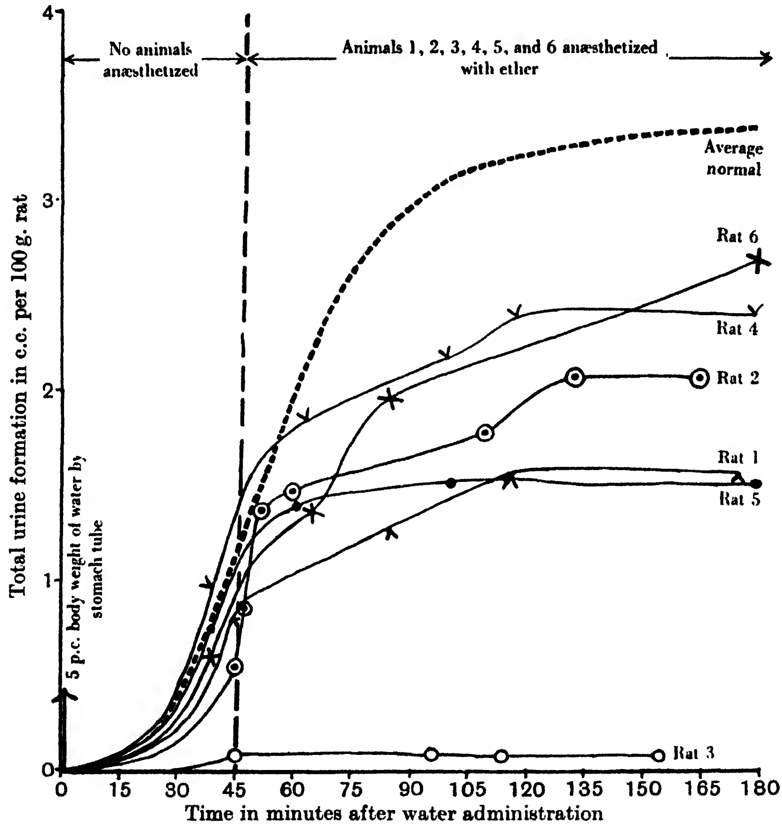

Fig. 5. The influence of ether anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

All rats used in these experiments were fed previously on bread and milk for a period of 3 days.

II. *The influences of chloroform and ether upon water diuresis when absorption is complete.* If water is given to an animal and a sufficient time for water absorption is allowed before subsequently administering the anæsthetic, it has been found that a marked inhibition of diuresis is still obtained. This is clearly seen on Figs. 4 and 5. where the diuresis in

each rat which was anæsthetized falls rapidly below the average normal output of the controls. With the exception that no anæsthetic was given the control rats were similarly treated. Figs. 6 and 7 give the average diuresis curves for the anæsthetized and normal rats and indicate the times at which the water absorption may be assumed to have taken place.

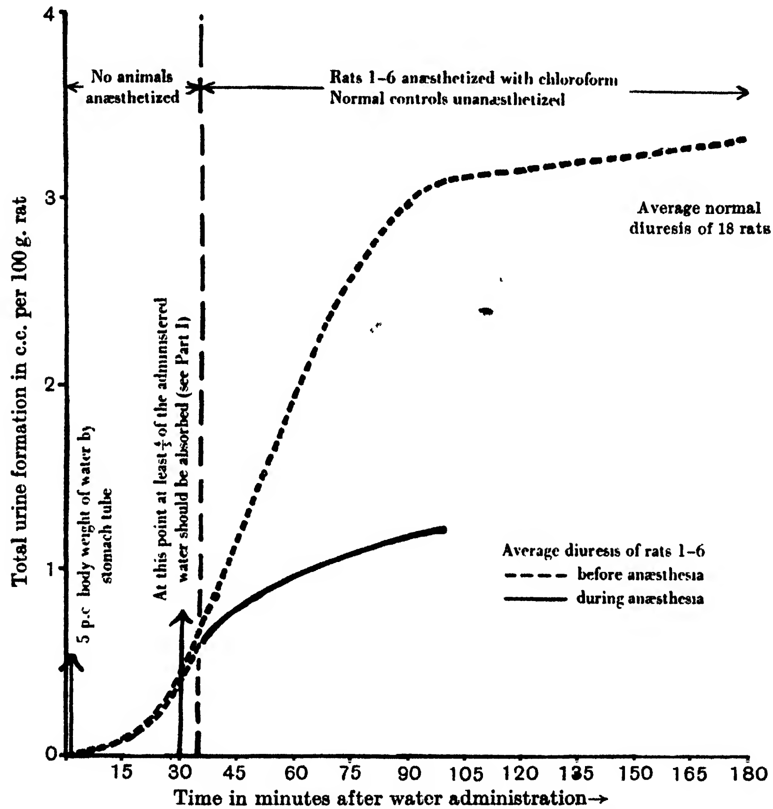

Fig. 6. The influence of chloroform anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

From Tables VIII and IX it will be seen that before an anæsthetic was given the average urinary outputs of the two sets of six animals is approximately equal to that of the eighteen normal controls. But during the period when the rats were anæsthetized with ether and chloroform respectively their urinary output fell markedly below that attained by the controls.

Thus at the end of 35 min. the average total urinary output was 0·62 c.c. per 100 g. rat for the normal controls and 0·60 c.c. for the animals which were to be anæsthetized subsequently.

In the next 65 min. the average total output of the normal controls was 2·48, but of the rats anæsthetized with chloroform only 0·61 c.c. per 100 g. rat.

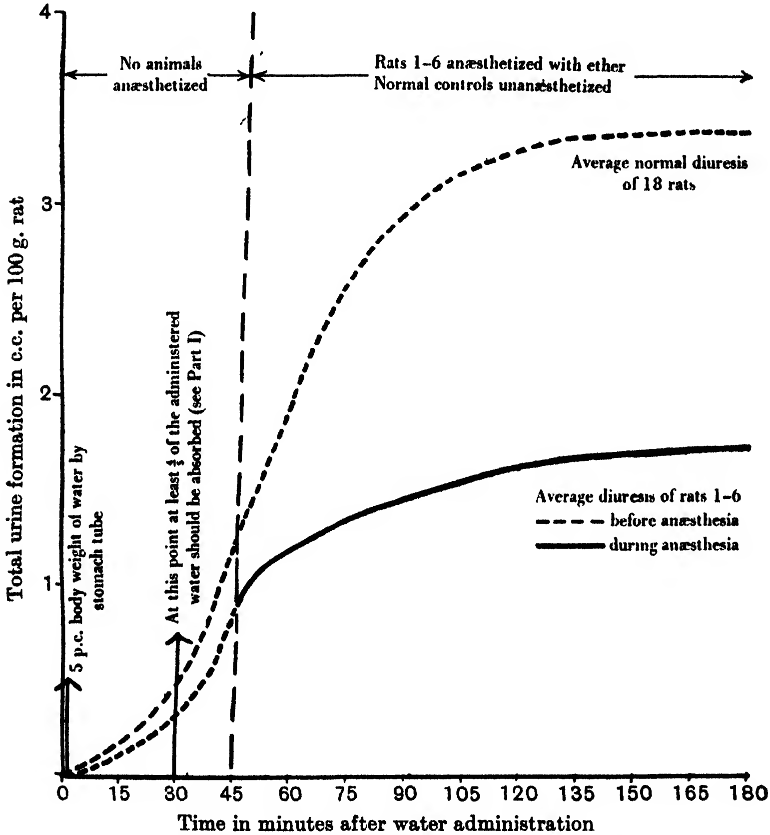

Fig. 7. The influence of ether anæsthesia upon water diuresis as distinct from its action upon alimentary water absorption.

Similar results were obtained using ether anæsthesia.

The water absorption curves for rats in which the previous treatment was similar and which were of the identical breed (Part I) show that water absorption is practically complete within 30 min. after the administration of a 5 p.c. of body weight dose of water by stomach tube.

TABLE VIII.

Time after water administration (min.)	Total urinary output in c.c. per 100 g. body weight of rats which were anæsthetized with ether 45 min. after giving water by stomach tube							Average total urinary output in c.c. per 100 g. rat of 18 normal controls (no anæsthetic given)
	1	2	3	4	5	6	Average	
20	0·13	0·09	0·00	0·26	0·22	0·17	0·15	0·23
40	0·45	0·30	0·04	1·00	0·80	0·66	0·54	0·93
45	0·78	0·54	0·08	1·37	1·10	0·93	0·80	1·17
Rats Nos. 1–6 now anæsthetized with ether								
50	0·92	1·20	0·08	1·57	1·22	1·08	1·01	1·43
60	1·04	1·46	0·08	1·79	1·37	1·26	1·17	1·95
80	1·23	1·58	0·08	2·00	1·47	1·81	1·36	2·75
100	1·42	1·70	0·08	2·17	1·50	2·10	1·49	3·10
120	1·53	1·88	0·08	2·38	1·50	2·26	1·61	—
140	1·53	2·06	0·08	2·38	1·50	2·41	1·66	—
160	1·54	2·06	0·08	2·38	1·50	2·54	1·68	—
180	1·54	2·06	0·08	2·38	1·50	2·67	1·71	3·35

TABLE IX.

Time after water administration (min.)	Total urinary output in c.c. per 100 g. body weight of rats which were anæsthetized with chloroform 35 min. after giving water by stomach tube							Average total urinary output in c.c. per 100 g. rat of 18 controls (no anæsthetic given)
	1	2	3	4	5	6	Average	
20	0·00	0·04	0·08	0·19	0·40	0·12	0·14	0·23
30	0·02	0·11	0·20	0·50	1·28	0·33	0·41	0·40
35	0·06	0·20	0·32	0·70	1·83	0·47	0·60	0·62
Rats Nos. 1–6 now anæsthetized with chloroform								
40	0·12	0·30	0·46	0·75	2·00	0·60	0·71	0·93
45	0·17	0·37	0·60	0·77	2·10	0·71	0·79	1·17
50	0·29	0·43	0·72	0·78	2·16	0·78	0·86	1·43
60	0·34	0·54	1·01	0·79	2·26	0·87	0·97	1·95
80	—	0·74	1·40	0·79	—	—	0·98	2·75
100	—	0·98	1·58	1·06	—	—	1·21	3·10
120	—	1·22	1·58	1·12	—	—	—	—
140	—	1·74	1·58	1·17	—	—	—	—
160	—	2·10	1·58	—	—	—	—	—
180	—	—	—	—	—	—	—	3·35

It is clear, therefore, that the results represent the influence of these anæsthetics upon the urinary output after absorption has taken place.

The extrarenal loss of water determined in rats anæsthetized with ether amounted to 3·55, 0·99, 2·97, 3·4, 1·16 and 3·38 per 100 g. rat in 3 hours. In two animals anæsthetized with chloroform it was 2·75 and 3·90 g. per 100 g. rat in 159 and 152 min. respectively. This is not in excess of normal.

The body temperature of the rats fell during anæsthesia, but for reasons described in a previous paper (Part II) it was thought inadvisable to apply heat externally. In any case the inhibition of diuresis takes place before there has been time for any appreciable loss of water or fall in temperature.

Discussion.

In rats the delay in intestinal absorption of water produced by ether and chloroform is so great that this alone would explain the absence of diuresis. But it has also been shown, that even when the induction of anæsthesia is postponed and time is allowed for water absorption there is still a marked inhibition of diuresis. The inhibitory actions of ether and chloroform are therefore twofold: on the alimentary canal delaying absorption and after absorption preventing excretion.

The experiments on rabbits suggest that anæsthetics may also tend to diminish the alimentary absorption in this animal.

In studying the inter-relationship of the nervous system and kidneys and in all diuresis experiments involving the administration of water by the alimentary canal it is clearly necessary to distinguish between enteral and renal phenomena. The parts played by these factors vary and must be separately assessed for each animal and anæsthetic used or a final interpretation of the results may be obscured.

SUMMARY.

In rats: (1) The alimentary absorption of water is delayed to a marked degree by ether and chloroform, to a slighter degree by luminal, paraldehyde and urethane and is not appreciably delayed by chloralose.

(2) Diuresis is inhibited by ether and chloroform even when adequate time for water absorption is allowed prior to the induction of anæsthesia.

(3) The inhibition of diuresis by ether and chloroform is not caused by any increased extrarenal water elimination.

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AN ANTI-GROWTH PRINCIPLE DERIVED FROM THE PARATHYROID GLAND.

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DURING the course of another investigation the existence in the parathyroid glands of a principle other than that controlling calcium metabolism was suggested. Moreover, preliminary experiments involving the injection of "parathormone" in rats over long periods demonstrated, among other things, some retardation of growth.

That the parathyroid glands play some part in body growth was further suggested by the somewhat sparse literature of this subject. Hansman [1930] has recently performed thyro-parathyroidectomy operations resulting in a marked gain in weight which, he maintains, is to be regarded as a manifestation of parathyroid imbalance, since the control experiments definitely rule out the possibility of these symptoms being due to hypothyroidism.

Hyperparathyroidism in children is reported as being associated with emaciation and decrease in body weight and failure to grow [Hurst and Cosin, 1931; Pemberton and Geddie, 1930].

It may also be of significance that the oxyphil cells of the parathyroid gland do not develop until the rate of growth begins to slow. In man they do not appear until the age of about adolescence, while hyperplasia of the parathyroid glands in aged individuals has been reported [Kurikawa, 1925].

Another fact possibly having some bearing on the subject is that parathyroid glands have not been found in fish and, as is well known, fish never cease to grow.

Therefore, the problem whether the parathyroid glands have some function relating to growth seemed worthy of investigation, and extracts of parathyroid glands free from the blood calcium raising factor (Collip) were prepared and the effects of their administration in animals observed.

The present paper presents the results thus obtained upon the growth rate of the animals. A preliminary paper has already been made by one of us (J. H. T.) [1931].

Methods of preparation.

A large number of preparations from ox parathyroid glands have been made. Many of these have been inactive in relation to animal growth, and most of them are not mentioned in this paper. The majority of the active extracts are modifications of the original method of preparation, details of which are given below. A fuller description of the methods of preparation and of the chemical properties of the extracts will be given elsewhere.

We are much indebted to Messrs Allen and Hanbury's, Ltd., for preparing many of these extracts, and for most generously supplying a large quantity of the original extract for the routine work.

Mr N. Evers has kindly supplied us with the following details of the methods of preparation of the extracts used for routine work.

Extract 1. 400 g. of parathyroid glands were freed from adhering fat, thoroughly minced and then mixed with a cooled mixture of 300 c.c. of concentrated sulphuric acid, 1800 c.c. of water and 900 c.c. of 95 p.c. alcohol. 700 c.c. of benzene were then added and the whole shaken for 48 hours. The gland residue was then strained off and the benzene separated from the liquor after the latter had been standing for some hours. The acid-alcoholic liquid was then neutralized by the gradual addition of strong sodium hydroxide solution until the *p*H value was about 4·8. Alcohol, 95 p.c., was then added to a concentration of 75 p.c. by volume and the mixture kept in a cool place for 16 hours to allow the sodium sulphate to crystallize out. The insoluble matters were filtered off and the filtrate was evaporated under reduced pressure to a volume such that 1 c.c. was equivalent to 2 g. of original gland tissue. The *p*H value of the liquid was adjusted to 4·8, and after allowing the product to stand until it no longer deposited it was filtered through a sterilizing Seitz filter.

Extract 2. Sodium sulphate was added to an extract made by the above process until the latter was saturated. The mixture was allowed to stand overnight and the salted out material was filtered off. It was redissolved in a smaller volume of water at *p*H 4·8 and again salted out. The combined filtrates were freed from sodium sulphate by the addition of alcohol to a final concentration of 75 p.c. by volume, cooled to about 5° C. and filtered at this temperature.

The alcohol was removed by distillation under reduced pressure and the volume of aqueous residue adjusted to its original value.

Extracts treated with hydrogen peroxide. 1 c.c of "100 volume" hydrogen peroxide solution was added to 300 c.c. of an active extract, the *p*H of which was about 4·8, and the mixture kept in an incubator at 22° C. overnight. The excess of hydrogen peroxide was removed by the addition of manganese dioxide which was filtered off when the required action had taken place.

Various fractions have been prepared of which a few are detailed below.

Extract No.	Method of preparation	Total solids p.c.	Ash p.c.
3	As extract 1 but heated with acid alcohol	7·4	1·3
4	Extract 3 treated with charcoal	—	—
5	Filtrate from treatment of extract 3 with 80 p.c. alcohol	—	—
6	Extract 5 treated with charcoal	—	—
7	Extract 3 treated with hydrogen peroxide	—	—
8	Precipitate obtained during evaporation of extract 5	2·50	0·65
9	Extract 3 autoclaved	8·0	1·70
10	Residue, from extraction with acid 80 p.c. alcohol, extracted with acid 30 p.c. alcohol	0·7	0·3
11	Extraction of acetone soluble matter from glands with acid alcohol	—	—
12	Alkaline extract of glands	6·0	0·55
13	Proteolytic digestion of glands	—	—
14	Proteolytic digestion of lean veal	—	—

All extracts used in this series of experiments have been free from all antiseptics, adjusted to *p*H 7, and are protein free.

Methods of administration. In all cases, unless otherwise stated, the extracts have been given by subcutaneous injection once daily.

An ordinary diet has been given to all animals, and in most instances the experimental animals and their controls have lived together in the same cages.

No local reaction has ever resulted from injecting the extracts detailed in this paper, although considerable reaction occurred with earlier extracts due to failure of adjusting the solution to *p*H 7.

RESULTS.

Retardation of growth has been successfully obtained in all the different kinds of animals used, viz. rabbits, rats, guinea-pigs, tadpoles and axolotls. The present paper deals with rabbits and rats. In no instance have any harmful effects been observed with the dosages used. No sickness, vomiting, diarrhœa, convulsions, nor any signs of hypercalcæmia have been observed. The animals have remained apparently healthy, feeding and exercising in a perfectly normal manner. Indeed, some slight beneficial effects have been noticed, the experimental animals being less subject to intercurrent disease.

Rabbits. Fig. 1 shows the average growth curves for the first 12 months of all the rabbits having one 5 c.c. dose daily, the dose used in this investigation thus far. As will be seen the growth rate of the experimental animals begins to be decreased about 4 weeks after the beginning of the injection of extract 1. Extract 2, however, induces an earlier retardation. With a 5 c.c. dosage growth is markedly retarded, and in some instances completely inhibited for a few months.

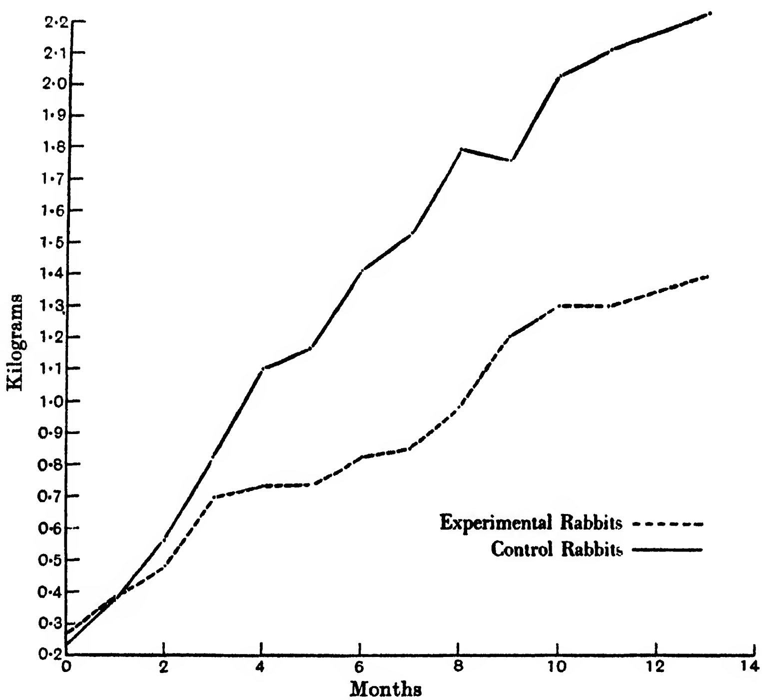

Fig. 1. Average growth curves of 26 rabbits injected with 5 c.c. of parathyroid extract daily, and of 10 control rabbits. It will be seen that at the end of 12 months there is a difference in weight of nearly 1 kg.

With smaller doses the degree of retardation varies more or less proportionately with the dosage as will be seen from Fig. 2.

Rabbits, inhibited in their growth rate by extract 1 (two of which are shown in Fig. 3), appear to develop after a few months a kind of tolerance to the extract. This is also obvious when using extract 2 over long periods of time, but the effect is a much smaller one. A few preliminary investigations show that when injections of extract 2 are

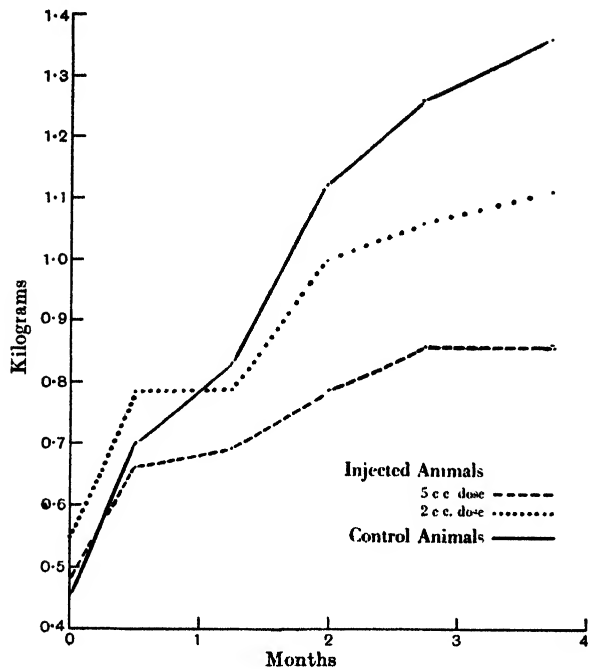

Fig. 2. Growth curves of rabbits of the same litter injected with 5 c.c. and 2 c.c. doses of parathyroid extract daily. The degree of growth retardation is approximately proportional to the dosage.

Fig. 3. Two rabbits of the same litter aged 9 months, the small one injected for 6 months with 5 c.c. parathyroid extract daily. Respective weights 1·29 kg. and 1·95 kg.

discontinued the experimental animals continue to grow at the retarded rate (Fig. 4).

The retardation of growth involves the whole body. The animals are well developed and normal except that they are much smaller in size than the controls (Fig. 3). That the growth of the skeleton is included can be seen from Fig. 5. No abnormality of the calcium metabolism has been found in the bone structure of any animals (Fig. 6). The control

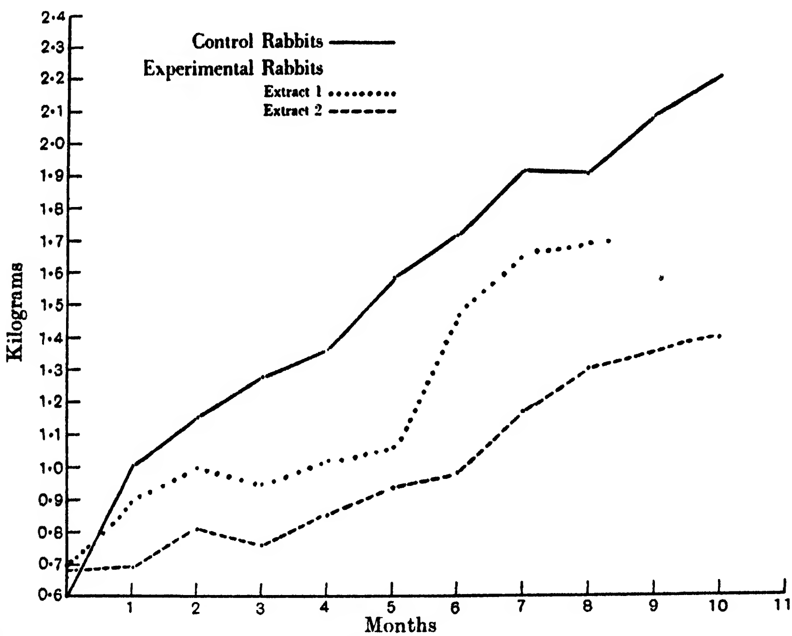

Fig. 4. Showing (*a*) the greater potency of extract 2 in retarding growth; (*b*) the tolerance developed to extract 1; (*c*) the continuation of the retarded growth rate after cessation of extract 2 at the seventh month.

rabbits have had equivalent doses of saline during the first 3 months, and equivalent doses of meat extract prepared in the same way as the parathyroid gland extract for the ensuing 2 months. As no effects upon the growth rate were obtained as a result of injecting it, its administration was discontinued.

More elaborate control experiments have been performed in the experiments on rats.

Rats. Extract 1 was found to be without much effect in rats. However, when more potent extracts were prepared results were readily obtained in them.

Fig. 5. Skeletons of rabbits shown in Fig. 3.

Fig. 6. Microphotograph of ground section of femur of rat injected with 6 c.c. of extract daily for 3 months to show that no decalcification of the bone has occurred.

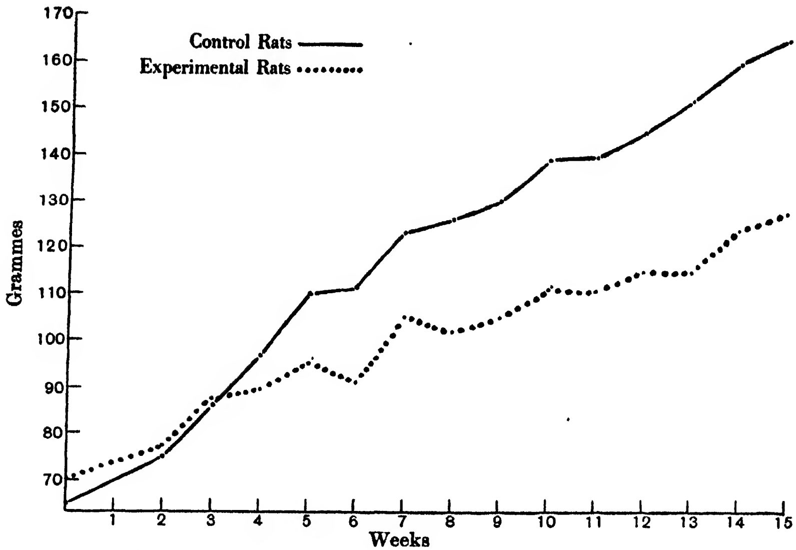

Fig. 7. Retardation of growth in rats by injection of 2 c.c. extract 2 daily. The curves represent the average weights of 24 rats.

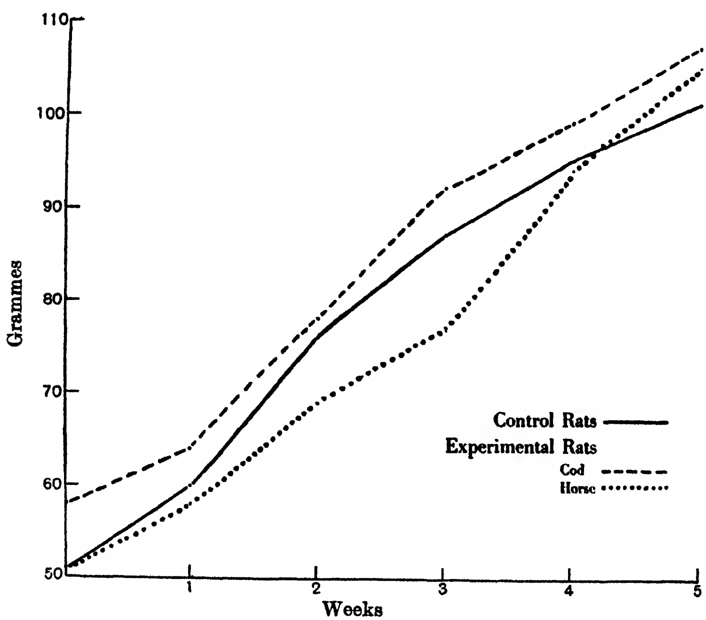

Fig. 8. The effects on growth of rats of extracts of horse muscle and of fish muscle made in a similar manner to the parathyroid extracts. 2 c.c. doses were injected. No variation from the normal growth was observed.

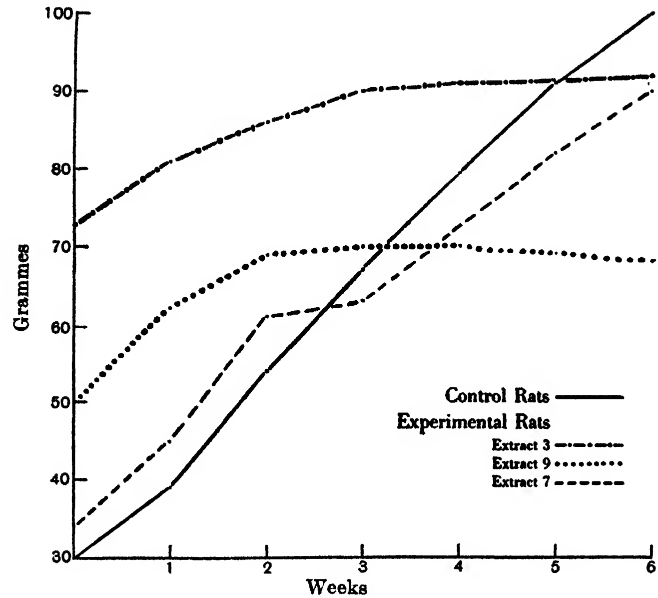

Fig. 9. Showing that autoclaving the extract does not affect its potency. The same extract, however, by washing with hydrogen peroxide has its activity destroyed.

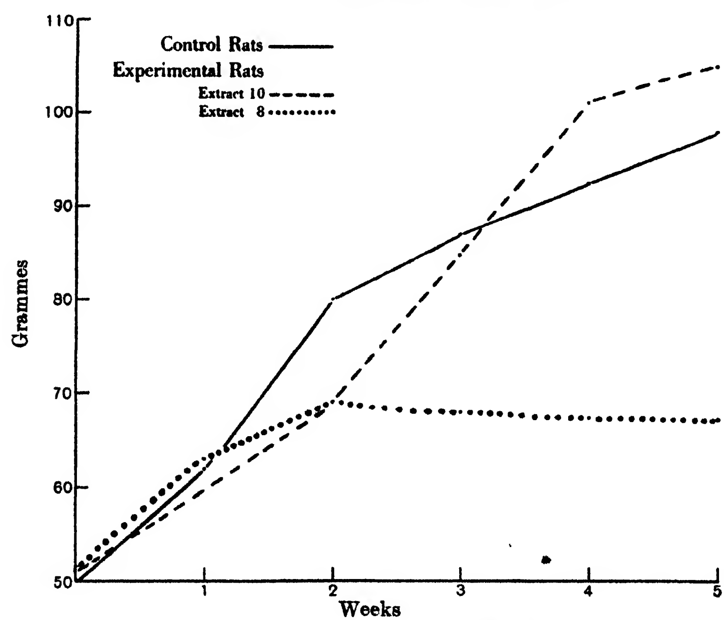

Fig. 10. The effects of injecting 2 c.c. doses daily of extracts 8 and 10 respectively upon rat growth.

Extract 2, freshly prepared, inhibits growth in rats by the end of 2 or 3 weeks (Fig. 7). As in the case of rabbits, the animals remain perfectly healthy during the period of retardation of their growth and exhibit no abnormal signs.

Control experiments. Extracts of various other tissues have been prepared by the same method of extraction, and given in equivalent doses to rats. Tissues so extracted include muscle (veal and beef), prostate gland, fœtus, and fish muscle. In no case has an inhibition of growth been obtained, but in some instances a definite acceleration of growth has resulted. Different methods of extraction yielding the anti-growth factor from the parathyroid glands have failed to elicit a principle from other tissues which has a similar effect on animal growth (Fig. 8).

Controls are also provided by inactive extracts of parathyroid glands or active extracts treated as indicated below.

Although we have so far failed to isolate the active principle, nevertheless some information has been obtained relating to its properties. For instance, boiling or autoclaving at 15 lb. for several hours the extract prepared according to extract 3 (see extract 9) does not affect its potency (Fig. 9). The extract, however, slowly loses its potency when kept at room temperature or even in a refrigerator.

On the other hand, washing the extract with hydrogen peroxide (extract 7) totally destroys all its activity (Fig. 9).

The active principle is apparently soluble in acetone, since acetone extraction of the glands yields a solution which inhibits growth in rats after a latent period of 2 weeks.

An equally potent preparation can be obtained from the precipitate formed during the evaporation of the filtrate resulting from treatment of extract 3 with 80 p.c. alcohol (see extract 8). This is of particular interest in view of the fact that it is probably the most potent fraction prepared and yet has a very low percentage of solids contained in it (Fig. 10). Extract 10 produced no effect on growth in rats (Fig. 10), but is mentioned because, in common with the other fractions retarding animal growth, it caused inhibition of plant growth. But its power to do so was rapidly destroyed by boiling. This fraction, therefore, seems to have in solution a thermolabile active principle which inhibits plant growth, but so far as we have been able to determine, is without effect on animal growth.

Discussion.

That the parathyroid glands play some rôle in growth is suggested by the observations referred to in the opening paragraphs. The difficulty in the past has been to dissociate this possible function from the well-known part which they take in regulating the calcium metabolism of the body.

No indication of any disturbance of the calcium metabolism has been observed in the experiments here recorded. Further investigation of this point is being pursued.

There is no evidence to indicate any toxicity of the preparations used. The total amount of solid matter present in the extracts is small, and it is significant in this connection that extract 8 with an extremely low solid content has a powerful inhibiting effect. None of the animals has shown any signs of toxæmia. Indeed, as already stated, many of the animals have been in slightly better physical condition than the controls. Injections daily extending over several months have failed to create any disturbance of normal health other than failure to grow. Indeed, it has been possible in several instances to keep animals constant in size and in weight for periods of 4 and 5 months.

The fact that some animals are more sensitive than others is difficult to explain. One of the difficulties encountered in reaching a conclusion is that no fully satisfactory method of extraction has been found. Extracts made in the same way vary in their potency, and occasionally are quite impotent as regards inhibition of the growth of rats. Whether this is due to some unknown factor in the extraction process, or whether the active principle has been destroyed or partially destroyed in the gland material prior to extraction we do not yet know. Nevertheless, since retardation of growth has been obtained in such a large number of animals, and no relation to any known substance in the extract or known function of the parathyroid has been demonstrable, we feel justified in concluding that the parathyroid glands contain an autacoid body with a growth-inhibiting function.

Summary.

Extracts of parathyroid glands have been prepared which have been injected into rabbits and rats.

Marked retardation of the growth rate has been demonstrated in most of the animals so treated, and reasons are advanced for concluding that an anti-growth factor exists in the parathyroid glands.

We desire to express our gratitude to Sir Arthur Keith and Prof. R. J. S. McDowall for much valuable advice and assistance, and to Mr W. MacCallum, without whose aid the earlier part of this work would have been impossible. We wish to thank too Dr W. Robson and Mr W. T. Boyd for kindly preparing some of the extracts.

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THE EFFECT OF PARATHYROID HORMONE AND OF IRRADIATED ERGOSTEROL ON CALCIUM AND PHOSPHOROUS METABOLISM IN THE RAT.

By L. I. PUGSLEY.

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I. Introduction.

The rat is commonly regarded as a species relatively refractory to the parathyroid hormone, although Tweedy and Chandler [1929] were able to show that continued large doses were lethal, and Rose and Stucky [1930] found that the serum calcium could be raised by two or three days' treatment. An increased calcium excretion has been observed by Waltner [1928], and very recently by Bülbring [1931], the calcium being presumably derived from the bones, which according to Waltner [1928] and to Lambie, Kermack and Harvey [1929] soon display osteoporosis. The object of the present research was to reinvestigate the action of parathyroid hormone in the rat, and to compare it with the action of large doses of irradiated ergosterol[1]. The latter is known to decrease calcium excretion in the fæces and increase it in the urine [Brown and Shohl, 1930; Watchorn, 1930] and the interpretation usually offered is that the absorption of calcium from the intestine is greatly increased, although according to Harris and Innes [1931] and others mobilization of calcium from the bones must also take place. Taylor, Weld, Branion and Kay [1931] affirm that in the dog the effects of overdosage with irradiated ergosterol are identical with those of parathyroid overdosage, and consider that in large doses vitamin D stimulates secretion from the parathyroid glands. It will be shown, however, that in the rat the metabolic effects of the two substances are entirely dissimilar, and recent studies in this laboratory by Selye [1932] prove that different histological changes are produced in the skeleton by these two agents.

The problem first investigated in this research was the effect of parathyroid hormone on the excretion of calcium and phosphorus in the urine and fæces. Greenwald and Gross [1926] found that the administration of parathyroid hormone to dogs led to an increased excretion of

[1] The writer is indebted to Eli Lilly and Co. for the parathormone and to Mead, Johnson and Co. for the ergosterol used in these experiments.

calcium in the urine, which was not maintained throughout the period of treatment and declined to subnormal levels in the recovery period; the phosphorus excretion in the urine followed a similar course, while the excretion of these elements in the fæces was little affected. These results have been confirmed by many workers, especially in studies of human subjects, in whom, according to Albright, Bauer, Ropes and Aub [1929] the rise in urinary calcium appears later than, and is probably secondary to, the rise in urinary phosphorus. Other references may be found in a recent review of the literature [Thomson and Collip, 1932].

II. General methods.

Albino rats of Wistar stock were used. They were kept in Hopkins' metabolism cages, and the urine and fæces were collected separately. In order to obtain sufficient urine for calcium analyses it was necessary to keep two rats (litter-mates) in a cage, and allow the urine to collect for 48 hours; the results, however, are expressed in mg. per rat per day throughout this paper. The diet used was McCollum's stock diet, except that no powdered bone was included, as it was felt undesirable to run the risk of contamination of the excreta with this ingredient; as compounded, the diet contained 0·266 p.c. phosphorus and 0·025 p.c. calcium. Male and female rats were used indifferently, and the animals weighed from 180 to 205 g. at the beginning of the experiments.

Analytical methods. The urine was ashed by a modification of the technique described by Kutz [1931] from this laboratory for dried tissue. It was evaporated to dryness in the 125 c.c. Pyrex Erlenmeyer flasks in which it was collected, and boiled for 3 or 4 hours with 3 to 5 c.c. of concentrated nitric acid (redistilled in glass to get rid of the traces of calcium usually present) until the digestion mixture was a clear straw-yellow; the nitric acid was then allowed to evaporate, and successive small amounts of nitric acid were added and evaporated until a white ash was obtained; the heating was carried out on a sand bath at about 200° C., and the whole process took about 24 hours. The ash was dissolved in HCl, calcium was determined on an aliquot portion by the method of Clark and Collip [1925], phosphorus on another portion (after heating with $10N$ H_2SO_4 to convert metaphosphate into orthophosphate, and to drive off excess HCl) by the method of Fiske and Subbarow [1925]. It was shown that known amounts of phosphate added to the urine could be quantitatively recovered by this method. The fæces were collected and dried in a silica dish and ashed in an electric muffle furnace at 500° C. for 3 hours; the ash was analysed by the method described above.

III. Experimental results.

The effect of parathyroid hormone upon the excretion of calcium and phosphorus by adult rats. The first problem studied was the effect of

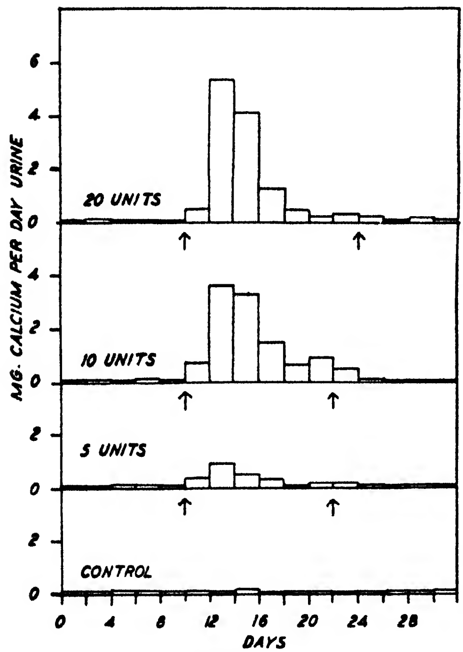

Fig. 1.

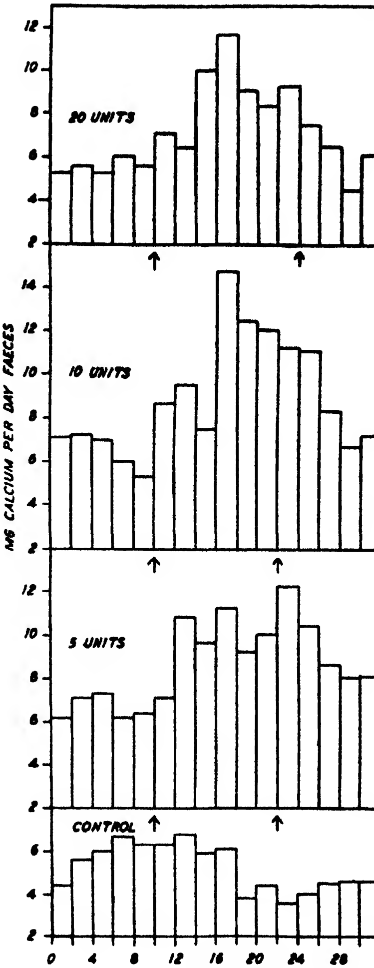

Fig. 2.

Fig. 1. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of calcium in the urine of adult rats.

Fig. 2. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of calcium in the fæces of adult rats.

varying amounts of parathyroid hormone ("Parathormone" Lilly) on the excretion of calcium and phosphorus. The experiments were divided into three periods (after the animals had become accustomed to the cages and the diet), during which the urine and fæces were collected from

each pair of rats every second day; a preliminary control period of 10–14 days was followed by a period of 12–14 days in which the extract was

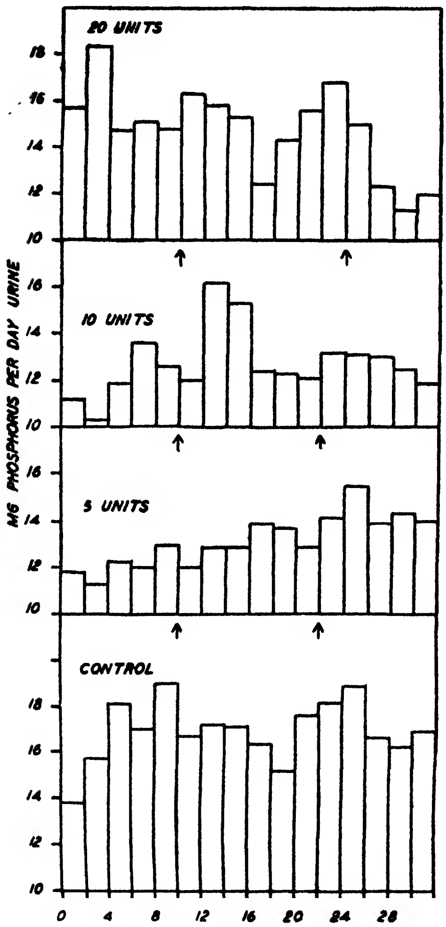

Fig. 3.

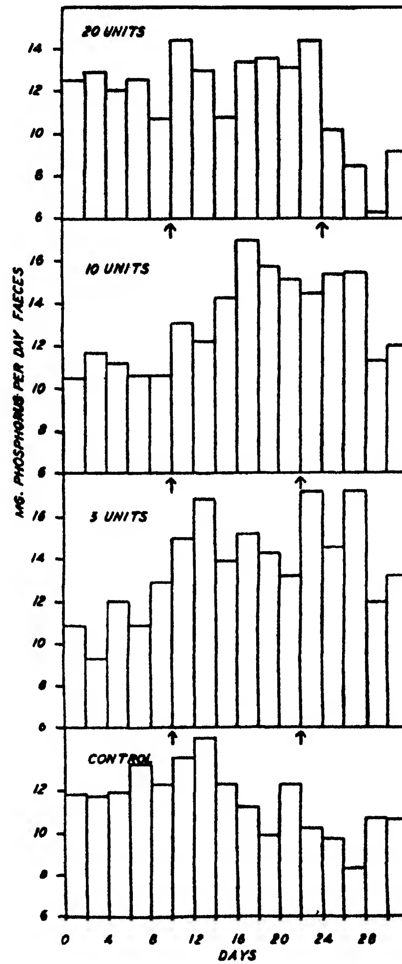

Fig. 4.

Fig. 3. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of phosphorus in the urine of adult rats.

Fig. 4. The effect of administration of 5, 10 and 20 units of parathyroid hormone daily upon the excretion of phosphorus in the fæces of adult rats.

injected subcutaneously in daily doses of 5, 10, or 20 units, and a recovery period. The results are shown in Figs. 1, 2, 3, and 4. Each curve gives the excretion per rat per day in mg. and each represents the

average excretion of three pairs of rats; three pairs of untreated controls were also followed. The most striking effects are seen in the increase in urinary calcium, which on the largest dose attains some fifty times the control value. It will be observed that the effect varies with the dose, and that it is not maintained throughout the period of treatment (the beginning and end of which are shown in the figures by arrows) but attains a peak on the fourth day and thereafter rapidly declines. In fact the rats appear to become immune rapidly or refractory to the extract, a phenomenon which has also been observed in dogs and in human subjects, and which is the object of further study in the later experiments. The calcium excretion in the fæces is also increased, but to a much less striking extent, in the period of treatment. Since the total "extra calcium" excreted, even on the largest dose, is not over 70 mg., it is not surprising that Day [1930] was unable to obtain significant reductions in the total body calcium of rats receiving much smaller doses of the hormone. The excretion of phosphorus in the urine is somewhat increased during the period of treatment, but the relative change is not nearly as great as that in calcium excretion, nor is there any evidence that it occurs more rapidly; the fæcal phosphorus does not appear to be significantly affected. None of these rats showed any ill-effects from the series of injections except a slight loss of weight; the administration of 40 units daily, however, was found to produce cachexia in 3 days and death in 5 or 6; the observation of Tweedy and Chandler [1929], that the congestion and hyperæmia of the stomach characteristic of dogs receiving excessive doses of parathyroid extract does not appear in rats, was confirmed by post-mortem examinations.

The effect of parathyroid hormone upon the serum calcium of adult rats. Blood was collected from rats by severing the carotid artery and jugular vein on one side and allowing the animals to bleed to death into a centrifuge tube; in order to obtain sufficient serum for the determination of calcium by the technique of Clark and Collip [1925], it was necessary to pool the blood from two rats. The average serum calcium of 40 pairs of rats on the diet described was found to be 10·3 mg. p.c., with a standard deviation of only 0·22 mg. p.c. Rats receiving 10, 20, or 40 units of parathyroid hormone daily were killed at various intervals and the serum calcium values obtained are plotted in Fig. 5, in which each point represents the average of determinations made on from three to eight pairs of rats. It is clear that marked hypercalcæmia can be produced, that it is to some extent proportional to the dose injected, and that it is not maintained throughout treatment but tends to return towards normal levels.

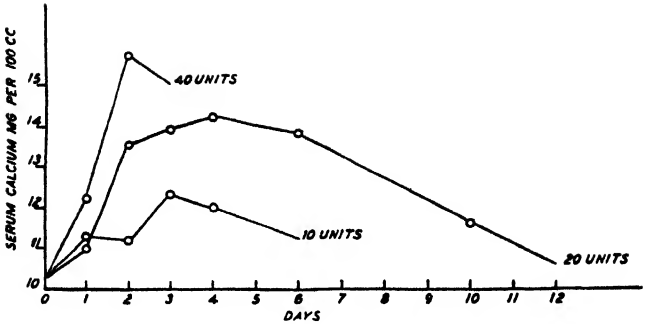

Fig. 5. The effect of administration of 10, 20 and 40 units of parathyroid hormone daily upon the serum calcium of adult rats.

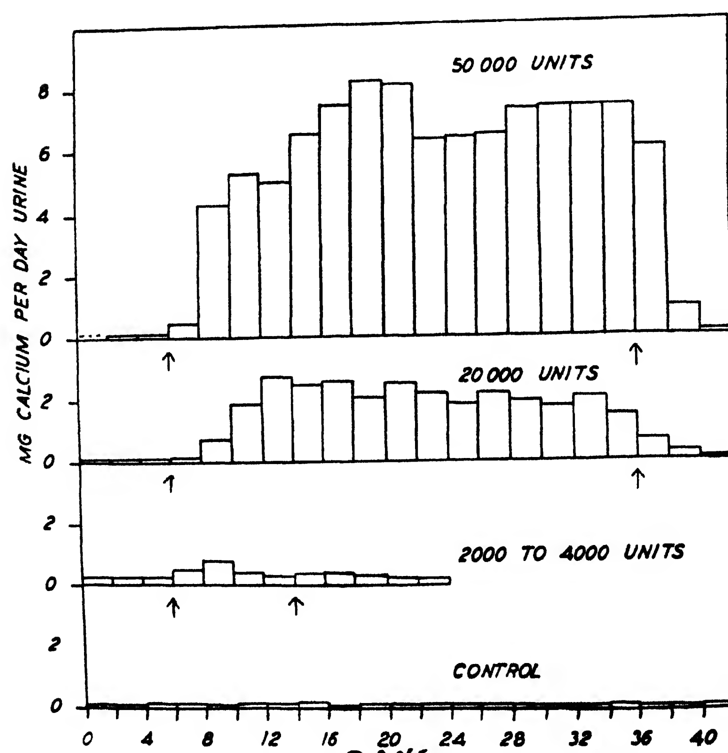

Fig. 6. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of calcium in the urine of adult rats.

The effect of irradiated ergosterol upon the excretion of calcium and phosphorus by adult rats. This experiment was carried out in the same manner as the first experiment. The irradiated ergosterol used was a sample prepared by Mead, Johnson and Co. for research purposes and was stated to have the potency $10{,}000\,X \pm 10$ p.c., assayed by the

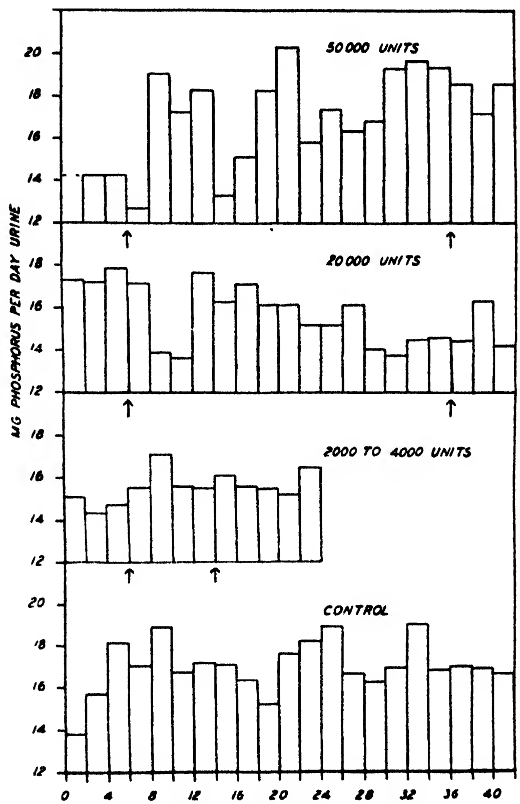

Fig. 7. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of phosphorus in the urine of adult rats.

method of Bills, Honeywell, Wirick and Nussmeier [1931]; according to the figures given by these workers, it therefore contained one million international rat units per c.c. Various dilutions in olive oil were prepared and were administered orally by means of a pipette in doses of 2000, 4000, 20,000 and 50,000 units daily, in each case in a volume of 0·1 c.c. per dose. The results of the experiments are shown in Figs. 6, 7, 8 and 9; as they are on the whole in harmony with those of other workers,

they do not require extended discussion. With the larger doses, there is a great increase in the excretion of calcium in the urine with no regular change in urinary phosphorus, and, in contrast to the experiments with

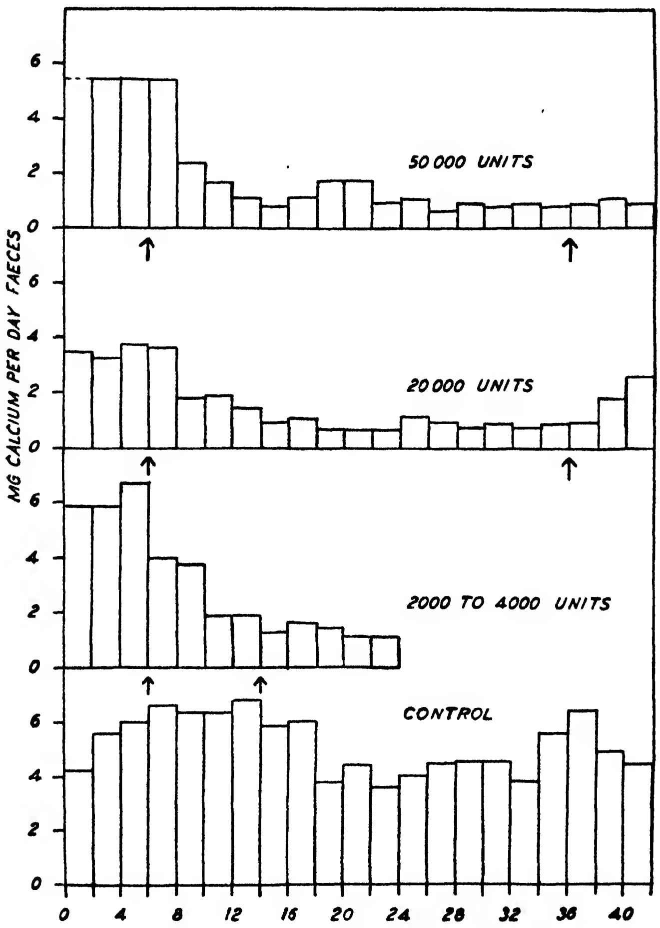

Fig. 8. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of calcium in the fæces of adult rats.

parathyroid extract, a very distinct decrease in the fæcal excretion of both elements. Except on the highest doses, the rats did not appear to suffer any ill effects from the treatment. These results are not readily brought into line with the theory that the chief effect of large doses of irradiated ergosterol is to stimulate the parathyroid glands.

The administration of irradiated ergosterol to rats refractory to parathyroid hormone. In this experiment a study was made of the calcium and phosphorus excretion of a group of rats which received first 10 units of parathyroid hormone daily for 12 days on two separate occasions, then

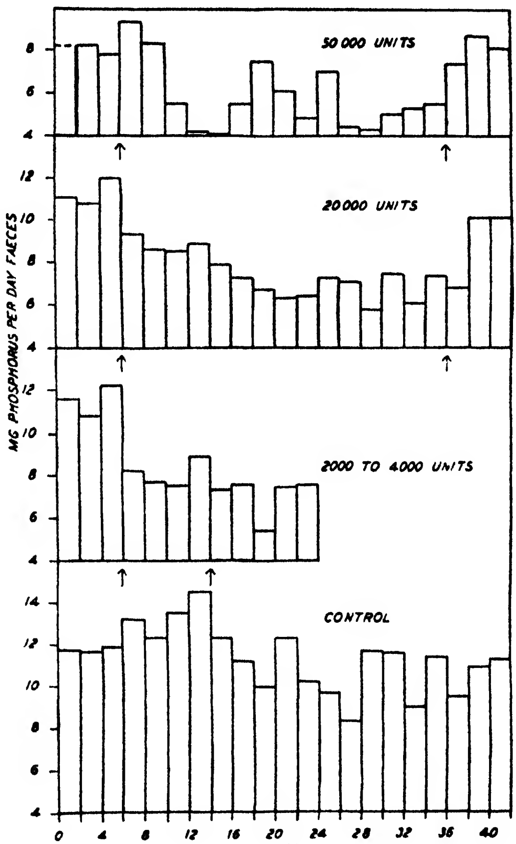

Fig. 9. The effect of administration of 2000 to 4000, 20,000 and 50,000 units of vitamin D daily upon the excretion of phosphorus in the fæces of adult rats.

received 50,000 units of irradiated ergosterol daily for 30 days, and lastly received 10 units of parathyroid hormone daily for 14 days. The results are shown in Figs. 10 and 11, in which the parathyroid periods are marked by a shaded block and the vitamin period by a black block. The urinary calcium increased in the usual way at the beginning of the first parathyroid period, but showed almost no response in the second and third parathyroid periods, though it increased as usual, with the usual

concomitant decrease in fæcal calcium, in the intervening vitamin period. This experiment shows firstly, that rats which have once become immune or refractory to parathyroid hormone do not readily regain sensitivity, and secondly, that such rats respond as usual to the treatment with irradiated ergosterol.

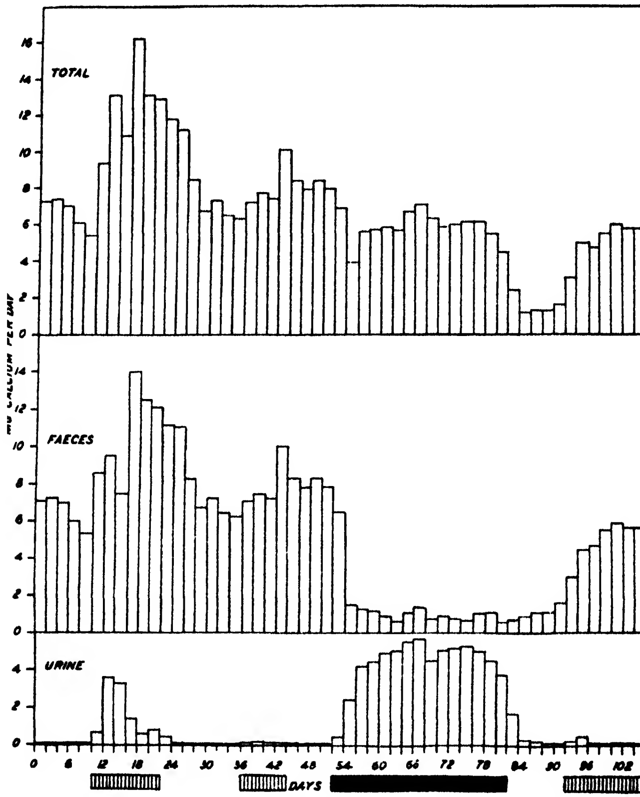

Fig. 10. Comparison of the effect of successive administration of parathyroid hormone with that of irradiated ergosterol upon the excretion of calcium of adult rats:

(hatched bar) 10 units of parathyroid hormone daily.
(black bar) 50,000 units of vitamin D daily.

The effect of urine of immune rats receiving parathyroid hormone on the calcium excretion of responsive rats. Since it was felt that the animals which had developed "immunity" to parathyroid hormone might be excreting the injected hormone in the urine, an investigation of the point was undertaken. Five pairs of rats which had previously received 10 units of parathyroid hormone daily in three periods, and which no longer

showed any response to the injections, were treated with 20 units of parathyroid hormone daily. One pair ("*A*") was kept as a control, the urine and fæces being analysed for calcium, which did not increase during the treatment. The urine of the other four pairs was injected into

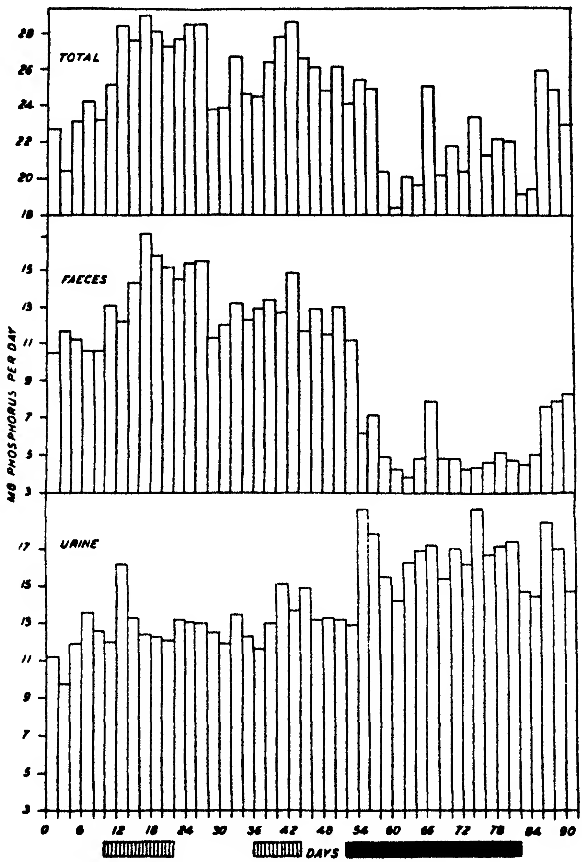

Fig. 11. Comparison of the successive administration of parathyroid hormone with that of irradiated ergosterol upon the excretion of phosphorus of adult rats.

three pairs of normal rats (*X*, *Y* and *Z*) whose calcium excretion was followed. The results are summarized in Table I. It is seen that though the amount of urine injected into each of the normal (and presumably responsive) rats daily might have contained 26·6 units of parathyroid hormone, if quantitative excretion occurred, and though the urine was collected in the presence of acetic acid and chloroform to prevent destruction of any hormone present, yet none of the rats receiving injections

TABLE I. Effect of administration of urine of rats immune to the action of parathyroid hormone, upon the excretion of calcium of normal adult rats expressed in mg. per day.

Date 1932	Urine: A Control	Urine: X	Urine: Y	Urine: Z	Fæces: A Control	Fæces: X	Fæces: Y	Fæces: Z	
Feb. 22	0·07	0·18	0·02	0·02	3·30	5·67	5·50	7·00	Control
„ 23	0·11	0·04	0·13	0·02	—	—	—	—	Experimental period
„ 24	0·07	0·15	0·17	0·23	3·30	4·75	5·70	7·40	
„ 25	0·15	0·04	0·04	0·04	—	—	—	—	
„ 26	0·12	0·14	0·08	0·29	4·90	5·37	5·37	7·70	

of this urine showed any significant increase in the excretion of calcium in the urine or faeces. It may be concluded that certainly not more than one-fifth of the parathyroid hormone injected into the immune rats appeared in the urine.

The effect of the injection of the serum of immune rats upon the response of normal rats to parathyroid hormone. It was also thought possible that in "immune" animals some substance antagonistic to the parathyroid extract might be present in the blood serum. Normal responsive rats were therefore treated with 20 units daily of parathyroid hormone and simultaneously with 2 c.c. of serum from either normal, responsive rats or previously treated "immune" rats, and the excretion of calcium in the urine of the injected animals was studied. The results are shown in Table II. Both groups of injected rats showed the typical great increase

TABLE II. Effect of administration of 20 units of parathyroid hormone and serum of rats immune to the action of parathyroid hormone, upon the excretion of calcium in the urine of normal adult rats.

Date 1932	Injected with normal serum + parathyroid hormone	Injected with immune serum + parathyroid hormone	
Jan. 3	0·15	0·07	Control period
„ 5	0·18	0·09	
„ 7	1·70	0·33	Experimental period
„ 9	4·80	5·93	
„ 11	2·44	1·25	
„ 13	0·39	0·39	
„ 15	0·30	0·38	

in urinary calcium during the first part of the period of parathyroid treatment, and neither normal serum nor "immune" serum inhibited this effect.

IV. DISCUSSION.

Although the rat is relatively refractory to parathyroid hormone, when compared with the dog or the man, nevertheless it is possible to produce an increased serum calcium and an increased excretion of

calcium in the urine and fæces by the injection of moderate doses. Of these effects, the greatest relatively is that upon the urine calcium in the first few days of treatment. Since this effect is obtainable with doses as small as 5 units daily per rat, and is roughly proportional to the dose, it suggests that a valuable method of biological assay of parathyroid extracts might be founded on this basis, the only objection being the tedious nature of the analysis required[1].

The apparent immunity to parathyroid extracts which the rats rapidly develop is of great interest. Attempts to demonstrate the presence of an immune body in the serum, or an increased permeability of the kidney to the injected hormone, completely failed. Since these experiments were carried out, however, an entirely new light on the nature of this immunity has appeared in the work of Selye [1932], in this laboratory. He has shown by histological study of the bones of rats receiving parathyroid extract, that while the first response is a formation of numerous osteoclasts with rarefaction of bone (doubtless leading to hypercalcæmia and increased calcium excretion), this is succeeded by the disappearance of the osteoclasts, the formation of numerous osteoblasts, with bone apposition proceeding to such an extent that the picture of "marble-bone disease" can be produced experimentally. In this second phase of the reaction one would expect to find a decrease in calcium excretion and possibly subnormal serum calcium.

The metabolic effects of large doses of vitamin D (irradiated ergosterol) are quite unlike those of parathyroid hormone. There is once more an increased excretion of calcium in the urine, but it is accompanied, as others have found, by a decreased excretion in the fæces; moreover, animals may be responsive to irradiated ergosterol when they are almost completely "immune" (as far as calcium excretion is concerned) to parathyroid hormone. It may be concluded that if large doses of irradiated ergosterol do stimulate parathyroid secretion, this is but a small part of their pharmacological action.

V. Summary.

1. General and chemical methods for following the excretion of calcium and phosphorus in the urine and fæces of rats are described.

2. The injection of parathyroid hormone, in doses of 5 to 20 units daily, leads to an increase in serum calcium, an increase in fæcal calcium

[1] After this article had gone to press F. J. Dyer reported before the Physiological Society, London, *J. Physiol.* **75**, 13 *P*, 1932, a method for the estimation of parathyroid hormone based on the rise in urinary calcium of male rats.

and urine phosphorus, and a relatively more striking increase in urine calcium, which however is not long maintained. Experiments on the nature of the apparent immunity to the hormone developed under treatment are described and discussed.

3. Oral administration of irradiated ergosterol, in doses of 20,000 to 50,000 units daily, leads to an increase in urine calcium but a decrease in fæcal calcium and phosphorus. This effect is also obtainable in rats which have become "immune" to parathyroid hormone.

I wish to express my thanks to Prof. J. B. Collip and to Dr D. L. Thomson for help and advice during the course of this work.

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INORGANIC SULPHATE EXCRETION BY THE HUMAN KIDNEY.

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IN putting forward his modern theory of renal function, Cushny [1917] pointed out that if any substance could be found which was eliminated entirely by glomerular filtration and was neither reabsorbed nor secreted by the cells of the renal tubules, then that substance could be employed to determine the actual rate of glomerular filtration. The truth of this can scarcely be questioned unless we suppose that the process occurring in the glomeruli is not one of simple filtration.

For thus estimating glomerular filtration rate, Cushny originally suggested that urea fulfilled the requirements, but later, in view of the accumulating evidence against an uncomplicated filtration of urea, abandoned that substance in favour of inorganic sulphate [1926].

Mayrs [1922] and White [1923] had already employed sulphate excretion for the same purpose in animals. In 1926 Rehberg pointed out the apparent relative advantages of creatinine excretion as an index of filtration rate, and in more recent papers has published observations which he interprets as supporting the view that creatinine fulfils the necessary requirements, *i.e.* is filtered off in the glomerular filtrate in the same concentration as in the plasma, and is neither reabsorbed nor secreted by the tubule cells. For any substance fulfilling these conditions the mode of calculation of the filtration rate is the same.

Thus if X_p and X_u be the concentrations of the substance in plasma and in urine respectively, expressed as mg. per 100 c.c., and V be the volume of urine excreted in c.c. per unit of time, then the glomerular filtration rate will be $100\frac{X_u . V}{X_p}$ c.c. in the same unit of time. This ratio, determined for creatinine, Rehberg calls the "glomerular filtration rate," but in the present paper it is considered preferable to employ the ratio $\frac{X_u . V}{X_p}$ and to call this the "clearance for substance X in keeping with the nomenclature now in frequent use in discussing urea excretion.

The terms "clearance for substance X" and "glomerular filtration rate as estimated by substance X" thus refer to essentially the same ratio, but differ in that the former attempts no interpretation of the physiological significance of the ratio, whilst the latter ascribes to it a meaning which as yet is not strictly justified. For this reason the former term is considered preferable.

Of the various substances occurring naturally in the urine, two, viz. creatinine and inorganic sulphate, have been held to afford reliable indices of the extent of glomerular filtration. If the claims for both substances are to be justified it must be shown that the results obtained by the use of creatinine are the same as those obtained with sulphate, and that this agreement holds under all conditions both physiological and pathological. In other words, the clearance for sulphate must always be the same as that for creatinine.

Accordingly Mayrs [1922] and White [1923] showed that when the blood sulphate concentration was raised to abnormally high levels by intravenous injection of sodium sulphate, the concentration ratios for creatinine and for sulphate were practically the same. Earlier technical difficulties in the accurate estimation of inorganic sulphate in the low concentrations, encountered in the plasma of normal animals and of men, prevented these observations from being extended to include the normal subject.

Nevertheless Poulsson [1930] sought to show that the two clearances were the same in man. He assumed the concentration of plasma inorganic sulphate in his experiments to lie within the limits of normal given by Wakefield [1929]. These limits were considerably below those obtained by other workers using different methods, and more recently Wakefield himself, in collaboration with Power and Keith [1931], and using an improved method, has obtained higher normal values in good agreement with those of other investigators. The deductions of Poulsson are thus based on figures which Wakefield himself has discarded as too low. It is clear therefore, that Poulsson's suggestion that the creatinine and sulphate clearances are of the same magnitude in man cannot be accepted on the evidence he provides.

In the present paper results of a number of direct determinations of the sulphate clearance in normal and in nephritic men are presented in which this claim of Poulsson could not be confirmed. The theoretical bearing of this fact is also considered.

METHODS.

Creatinine in urine was determined by the usual method of Folin [1914].

Creatinine in plasma was determined colorimetrically in a Folin-Wu filtrate. Details of the precautions employed have been given elsewhere [Cope, 1931 *a*].

Sulphate in urine. The gravimetric method of Folin [1905] was found entirely satisfactory and was used throughout.

Sulphate in plasma and serum. This has been estimated by two entirely independent methods: (i) Nephelometrically, by a modified Denis technique giving an accuracy of about 10 p.c. [Cope, 1931 *b*]. (ii) By benzidine precipitation and microtitration [Cope, 1931 *b*], a method in which the error does not exceed 5 p.c. in the concentrations estimated.

In the tables of results these methods are designated by the letters N and B respectively.

Urea in blood and in urine was determined by the urease and aeration methods of Van Slyke and Cullen [1914].

RESULTS.

Table I shows the results of a series of inorganic sulphate clearance determinations made on healthy young adults, in which the nephelometric method of serum sulphate estimation was used. Observations were made during the morning hours on subjects who had had no breakfast and who were during the observational period moving about the laboratories. The condition was, therefore, not one of complete rest, nor was a diuresis provoked. Urine was collected over a period of one hour, in the middle of which a sample of venous blood was taken for sulphate analysis in the serum.

The results are divided into two groups. In the first, plasma sulphate concentration was normal; in the second it had been raised somewhat by the oral ingestion of 10 g. of sodium sulphate the previous evening. It will be seen that, with one exception, the sulphate clearances lie between 26·0 and 51·3, with a mean value for each group of about 35·5.

Although, unfortunately, simultaneous creatinine clearance determinations were not made in this series, there can be little doubt that these would be considerably higher than 35·5. Holten and Rehberg [1931] give a minimum normal value of about 60 with a mean of about 90.

TABLE I. Sulphate clearances in normal human subjects. (Nephelometric method.)

A. Normal plasma sulphate concentration.

Subject	Volume of urine per hour	Urine inorganic sulphate mg. S per 100 c.c.	Inorganic sulphate excretion mg. S per hour	Serum inorganic sulphate	Sulphate clearance (hourly)
Hunt	39·5	99·00	39·00	1·11	35·4
Osborn	51·5	50·86	26·20	0·72	36·1
Osborn	86·0	46·50	40·00	0·78	51·3
Cope	39·0	61·60	24·00	0·80	30·0
Palin	47·5	107·00	50·88	1·15	44·2
Cope	41·2	55·25	22·76	1·17	19·5
Starling	18·5	284·80	52·68	1·60	32·9
			Mean	1·05	35·6

B. 15–18 hours after oral ingestion of sodium sulphate (10 g.).

Subject	Volume of urine per hour	Urine inorganic sulphate mg. S per 100 c.c.	Inorganic sulphate excretion mg. S per hour	Serum inorganic sulphate	Sulphate clearance (hourly)
Osborn	57·0	118·60	67·64	1·76	38·2
Talbot	61·0	115·30	70·30	1·76	40·0
Cope	54·0	160·60	86·90	1·87	46·6
Watson	27·0	249·20	67·05	1·92	34·9
Palin	38·0	156·70	59·57	2·24	26·6
Bosworth	189·0	39·48	74·63	2·26	33·0
Cope	46·0	133·60	61·45	2·27	27·0
Swan	66·1	134·10	88·52	2·42	36·3
			Mean	2·06	35·3

Poulsson [1930] obtained creatinine clearance values from 80 to 108 per hour, and the present writer has also obtained similar figures.

A series in which simultaneous determinations of urea, creatinine and sulphate clearances were all made on the same subject is shown in Table II. With the exception of (10 *a*) and (11 *a*), these determinations were all made with the subject at rest, and in the presence of a large diuresis provoked by combined water and urea ingestion, *i.e.* the so-called Addis conditions.

That such conditions do not materially influence the sulphate clearance is indicated by experiments (10 *a*) and (11 *a*) which were performed on the same mornings as (10) and (11) respectively, but during moderate exercise and in the absence of diuresis. Here a very considerable change in conditions has had no significant effect on the sulphate clearance. In this series the sulphate clearance, far from equalling the creatinine clearance, seldom exceeds one-third of the value of the latter, and tends, indeed, to be slightly lower than the maximum urea clearance. That the same essential ratio between the three clearances tends to persist when the functional activity of the kidney is reduced by disease, is shown in the observations on nephritic human subjects given in Table III.

TABLE II. Comparison of urea, sulphate and creatinine clearances in human subject C. L. C.

No.	Creatinine clearance: Un-corrected	Creatinine clearance: Corrected	Urea clearance (hourly)	Volume urine per hour	Urine inorganic sulphate mg. S per 100 c.c.	Excretion inorganic sulphate mg. S per hour	Plasma sulphate mg. S per 100 c.c.	Inorganic sulphate clearance (hourly)	Method
1	59·3	90·8	—	662	6·21	41·1	1·34	30·9	N
2	66·9	113·3	—	656	5·17	33·9	0·97	34·8	N
3	101·0	121·0	42·2	478	7·31	34·9	0·90	38·8	N
4	120·2	135·1	45·0	564	6·48	36·6	1·14	32·1	N
5	119·4	137·7	42·1	442	7·87	34·8	1·34	25·9	N
6	100·5	120·8	36·0	438	7·48	32·8	1·78	18·4	N
7	100·0	115·0	35·0	734	3·98	29·3	1·11	26·4	B
8	109·2	125·0	—	490	8·28	40·56	1·18	34·4	B
9	104·6	117·5	—	820	4·35	35·64	1·00	35·6	B
10	110·0	125·0	—	667	4·96	33·08	0·905	36·5	B
10 *a*	115·4	126·0	—	83	40·1	33·23	0·88	37·8	B
11	107·0	113·0	—	748	4·24	31·7	1·01	31·4	B
11 *a*	97·8	109·5	—	40·6	94·4	38·3	1·11	34·6	B
Mean	—	**119·2**	**40·0**	—	—	—	**1·13**	**32·1**	—

Note. The letters N and B in the last column indicate respectively the nephelometric and benzidine methods of estimating plasma sulphate.

Corrected creatinine clearances are obtained by subtracting 0·5 mg. from the estimated plasma creatinine value and recalculating the ratio [see Cope, 1931 *a*].

TABLE III. Comparison of sulphate clearances with urea and creatinine clearances in nephritis.

No.	Maximum urea clearance	Un-corrected creatinine clearance	Corrected creatinine clearance	Volume of urine per hour	Plasma inorganic sulphate mg. S per 100 c.c.	Urinary sulphate mg. S per 100 c.c.	Excretion inorganic sulphate mg. S per hour	Sulphate clearance (hourly)	Method
1	21·9	59·5	67·3	105	0·525	13·3	14·0	26·6	B
2	35·6	95·0	110·0	670	1·87	8·99	41·5	22·2	N
3	—	69·9	78·2	471	3·14	13·96	65·8	20·9	N
4	27·1	29·0	52·7	424	2·90	14·15	60·0	20·7	N
5	20·0	59·9	65·4	124	2·44	40·3	50·0	20·5	B
6	—	56·8	61·7	296	1·67	11·12	32·6	19·6	B
7	—	55·2	60·1	114	2·47	40·3	45·9	18·6	B
8	—	74·2	81·3	166	0·99	10·0	16·7	16·8	B
9	22·0	39·6	52·3	184	1·90	15·4	28·3	14·9	N
10	17·2	41·2	45·4	190	2·44	19·06	36·2	14·8	N
11	—	78·5	88·8	148	0·82	8·22	12·2	14·8	B
12	21·3	54·9	62·4	128	1·04	10·22	13·1	12·6	B
13	4·47	11·9	12·85	50	5·80	93·1	46·5	8·0	N
14	6·53	14·3	17·7	61	5·00	53·7	32·6	6·5	N
15	4·07	7·6	8·14	61	8·00	52·2	32·0	4·0	B
16	—	—	—	25	16·00	151·8	37·9	2·27	N

In the final column the letters N and B indicate respectively the nephelometric and benzidine methods of estimating plasma inorganic sulphate.

Discussion.

The important point which it is desired to stress is that the value of the sulphate clearance in the human subject, healthy or nephritic, is consistently below that for the creatinine clearance, and is usually only about 30 p.c. of the latter. In no case has the sulphate clearance even approached the value for the creatinine clearance, and this remains true even if uncorrected creatinine clearances are considered.

It is evident therefore, that both substances cannot be regarded as indices of glomerular filtration rate.

Several alternative explanations of the difference are possible. If it be supposed that the creatinine clearance does indeed represent the glomerular filtration rate, then we must conclude either that about two-thirds of the plasma inorganic sulphate exists in the circulating blood in a non-diffusible form, or else that a similar fraction is reabsorbed in the tubules. The former possibility is unlikely since blood may be completely freed of its inorganic sulphate by dialysis through collodion. It must be conceded, however, that as this process takes some considerable time, the possibility that an indiffusible compound is gradually converted into a diffusible form during dialysis cannot be definitely excluded.

The assumption that two-thirds of the sulphate filtered in the glomeruli is reabsorbed during its passage down the tubules, also presents difficulties, for if it were so, it would be reasonable to suppose that the induction of a heavy diuresis, by affording less opportunity for reabsorption, would lead to an increase in the sulphate excretion rate and so in the sulphate clearance. Such an increase apparently does not occur.

Moreover, the fact reported by Mayrs [1922] and by others, that ureteric obstruction reduces urea excretion much more than it does that of sulphate, has been interpreted by holders of the filtration-reabsorption view as evidence that urea is more readily reabsorbed than is sulphate. If this conclusion be true, then the sulphate clearance should be higher than that for urea. For although maximal urea clearances are being considered, the reabsorption theory must suppose that even from such dilute urines 50 p.c. or more of the filtered urea is reabsorbed, in order to account for the fact that the creatinine clearance is so much higher than the urea clearance.

Yet in the instances here reported, the sulphate clearance tends rather to be lower than the urea clearance, and the experience of Sager [1930] in dogs would appear to be similar, for his results show sulphate clearances sometimes slightly below, and sometimes rather above the urea

clearances. Furthermore, if we postulate such a large reabsorption of sulphate then it is necessary to suppose that through the tubule cells sulphate escapes with ease but creatinine with extreme difficulty, although into other tissues of the body creatinine is readily diffusible whilst sulphate only passes with great difficulty [Denis and Leche, 1925].

If, however, the possibility of secretion into the lumen through the tubule cells be conceded, then the explanation becomes relatively simpler. We may now suppose that the actual glomerular filtration rate is equal to, or somewhat less than, the value indicated by the average sulphate clearance, *i.e.* about 3·5 litres per hour in the normal human adult. Let us assume that the actual glomerular filtration be only 3 litres per hour. This will correspond to an hourly glomerular clearance activity of 30 for both creatinine and sulphate. If, further, we assume that the sulphate from 500 c.c. of plasma and the creatinine from 9 litres of plasma are eliminated through the tubules, then the observed values can be accounted for, for these figures correspond to tubular clearing activities of 5 and 90 respectively, bringing the total clearing activity of glomeruli and tubules combined, *i.e.* of the kidneys as a whole, up to 35 and 120 respectively. Such a view requires a behaviour of sulphate and of creatinine towards the tubule cells which is similar to that shown towards other tissues, and consequently would appear preferable to the filtration-reabsorption view which necessitates relative rates of passage of these substances through the tubule cells which are the reverse of those through other tissues of the body.

Rehberg bases his hypothesis that creatinine is filtered off, but neither reabsorbed nor secreted in the tubules, upon the following points [Holten and Rehberg, 1931].

(i) Its concentration ratio is higher than that of any other substance.

(ii) The excretion rate is independent of the volume of diuresis.

(iii) Change in osmotic pressure of the plasma proteins is associated with a change in rate of creatinine excretion in the same direction as would be anticipated if the excretory process were one of ultrafiltration in the glomeruli.

Of these, the high concentration ratio and the constancy of excretion with changing urine volume are equally compatible with a secretory mechanism. The relation to plasma protein osmotic pressure, whilst affording evidence suggestive that some creatinine is filtered off in the glomeruli, cannot be taken as evidence that all the creatinine is excreted in this manner.

In a recent study of the action of cyanide on the isolated kidney, Bayliss and Lundsgaard [1932] observed a very marked drop in the creatinine clearance. Being unwilling to believe that the glomerular filtration rate really fell to such an extent under the influence of cyanide alone, these writers postulated a progressively increasing leakage of fluid and of creatinine outwards from the tubule lumen. Their results, however, are equally compatible with the view that cyanide inhibits a creatinine secretory mechanism in the tubules, so that at the end of the experiment only that fraction of the creatinine which is filtered off in the glomeruli appears in the urine. Whether simultaneously any reduction in the true filtration rate did occur in their experiments can scarcely be decided, but it would seem probable.

More direct evidence pointing to a secretory elimination of creatinine is contained in the experiments of Edwards and Condorelli [1928] on the aglomerular kidneys of certain fishes. Nor can the similarity of behaviour of phenol red and creatinine during excretion which was shown by Marshall and Kolls [1919] be brought forward as suggesting a filtration, for although Oliver and Shevky [1929] present evidence that phenol red is eliminated solely by glomerular filtration, yet Höber [1930] maintains that it is secreted by the tubules, and the more recent work of Chambers on the behaviour of tissue cultures of embryonic tubules suggests strongly a secretory mechanism for the dye.

As regards the mechanism of excretion of inorganic sulphate, the situation is scarcely more clear. White [1923], from his experiments on the concentration ratios, concluded that sulphate comes in part through the glomeruli and in part through the tubules by secretion. Starling and Verney [1924] interpreted the fall in rate of excretion and in concentration of sulphate in the urine from a kidney poisoned by cyanide, as evidence of an active secretory process. Such an interpretation was, however, based on the belief that cyanide had no effect on the volume of glomerular filtrate, an assumption which must be considered doubtful in view of the work of Höber and Mackuth [1927] and of Bayliss and Lundsgaard [1932].

Of the attempts to dissociate glomerular from tubular elimination of sulphate in the frog, the earlier ones of Cullis [1906] and of Atkinson, Clark and Menzies [1921] must be interpreted with caution in view of the possibility of access of the tubular perfusion fluid to the vessels of the glomerular system. In the experiments of Yoshida [1924], this danger would appear to have been lessened. Yoshida found that when sulphate was supplied to the glomeruli of the frog's kidney it was well excreted and

concentrated, but that when applied to the tubules alone it appeared in the urine only in traces. More recently again, Kawasoe [1930], also working on frogs, has stated his conclusions that sulphate is excreted mainly by the glomeruli and possibly also slightly by the tubules. He also believed that no significant reabsorption of sulphate occurred.

Such results as these would thus agree well with the view presented here that the glomerular filtration rate is equal to, or somewhat less than the figure indicated by the sulphate clearance.

It seems to the writer highly desirable that this possibility should not be lost sight of. There is, strictly speaking, at present no real justification for supposing that the creatinine clearance is a more reliable index of glomerular filtration than is the sulphate clearance. There has of more recent years been a tendency to forget that Rehberg's suggestion was admittedly no more than a hypothesis based on an assumption concerning the behaviour of creatinine in the kidney. The rival claims of sulphate as a filtration index would seem to be at least equally strong. Possibly neither is a true index.

Whilst this work was being prepared for the press, there appeared a report of similar work arising from the same line of thought by Hayman and Johnston [1932]. Using a different method of estimating plasma sulphate, they obtain essentially the same results. They find the concentration ratio for inorganic sulphate much below that for creatinine, and in most cases also below that for urea. With such results those presented here are in complete agreement. These workers interpret their findings as evidence of back diffusion in the tubules, a conclusion which, as pointed out above, the present writer considers the less likely possibility.

Summary.

The excretory activity for inorganic sulphate of the human kidney, either healthy or nephritic, is only about one-third of that for creatinine.

Calculations of the glomerular filtration rate based on the excretion of inorganic sulphate thus give a value only about one-third of that indicated by the excretion of creatinine.

The theoretical consequences of this fact are considered.

This work was done under the tenure of a Beit Memorial Fellowship for Medical Research, and constitutes part of that submitted in a Thesis for the degree of Doctor of Medicine in the University of Oxford in 1931.

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577.174.5:612.133

A STUDY OF THE INFLUENCE OF ADRENALINE ON THE SYSTEMIC BLOOD FLOW.

BY HENRY BARCROFT,

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(*From the Physiological Laboratory, Cambridge.*)

THE object of this paper is twofold. Firstly to describe the changes in the systemic flow in dogs after the injection of adrenaline. Secondly to demonstrate certain phenomena which adrenaline brings about.

The systemic output has been measured with a mechanical stromuhr. This instrument has allowed a more complete study of the systemic flow under the influence of adrenaline than has been hitherto undertaken. The instrument and the accuracy of its readings have been fully described in a previous paper [Barcroft, 1929].

I. *A description of the changes in the systemic flow observed after adrenaline injection.*

Ten separate experiments have been performed. Each dog weighed approximately 10 kg. The operative procedure necessary for diverting the systemic flow through the mechanical stromuhr is fully described elsewhere [Barcroft, 1931 *a*]. The preparation is shown diagrammatically in Fig. 1 *a*. The position of the stromuhr and of the injections of adrenaline are indicated. The injections were made either intravenously or into the aorta peripheral to the coronary arteries but central to all other branches. Each injection contained from 0·05 to 0·1 mg. of adrenaline. The action of adrenaline on the vascular system was studied after section of the vagi. In some experiments the brain was destroyed by temporary obstruction of its blood supply; this did not affect the variations in the systemic flow seen after adrenaline.

Figs. 2, 3, 4 and 5 show variations in the systemic flow, arterial blood-pressure and heart rate in response to adrenaline. They illustrate the following:

(1) Figs. 2 and 3 are drawn from data supplied by one experiment. They show that whether the adrenaline was injected intravenously or

into the aorta the systemic flow varied, but was not much increased or decreased.

(2) Figs. 4 and 5 are drawn from data supplied by another experiment. They show that whether the adrenaline was injected intravenously or into the aorta the systemic flow varied and was greatly increased.

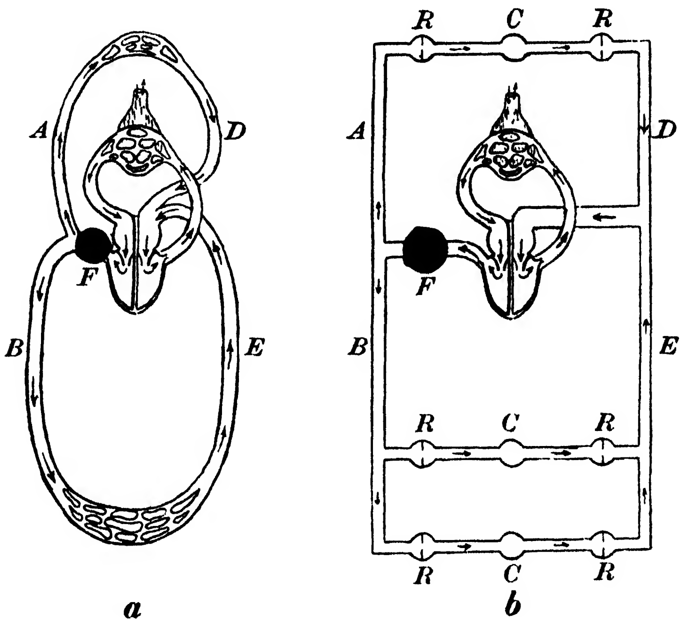

Fig. 1.

a. Diagram of animal's vascular system: *A*, brachiocephalic artery; *B*, thoracic aorta; *D*, superior vena cava; *E*, inferior vena cava; *F* represents the position of the stromuhr; *F* represents the position of the aortic injections of adrenaline; *F* represents the point at which the screw clamp was applied; *E* represents the position of the injections of adrenaline into the femoral vein.

b. Diagram of the cardio-pulmonary system attached to the artificial peripheral vascular system: *A*, tube representing the brachiocephalic artery; *B*, tube representing the thoracic aorta; *D*, tube representing the superior vena cava; *E*, tube representing the inferior vena cava; *R*, resistance; *C*, extensible part of peripheral vascular system; *F* represents the position of the stromuhr.

In most experiments adrenaline did not greatly increase the output, but all grades between the two extremes illustrated have been observed. In Figs. 4 and 5 the initial blood-pressure is seen to have been very low, but a large increase in the systemic flow has been seen in animals with a normal blood-pressure.

The curves also show:

(3) The systemic flow is at first increased by intravenous injections. This increase is coincident with a rise in the heart rate and in the arterial blood-pressure (Figs. 2, 4).

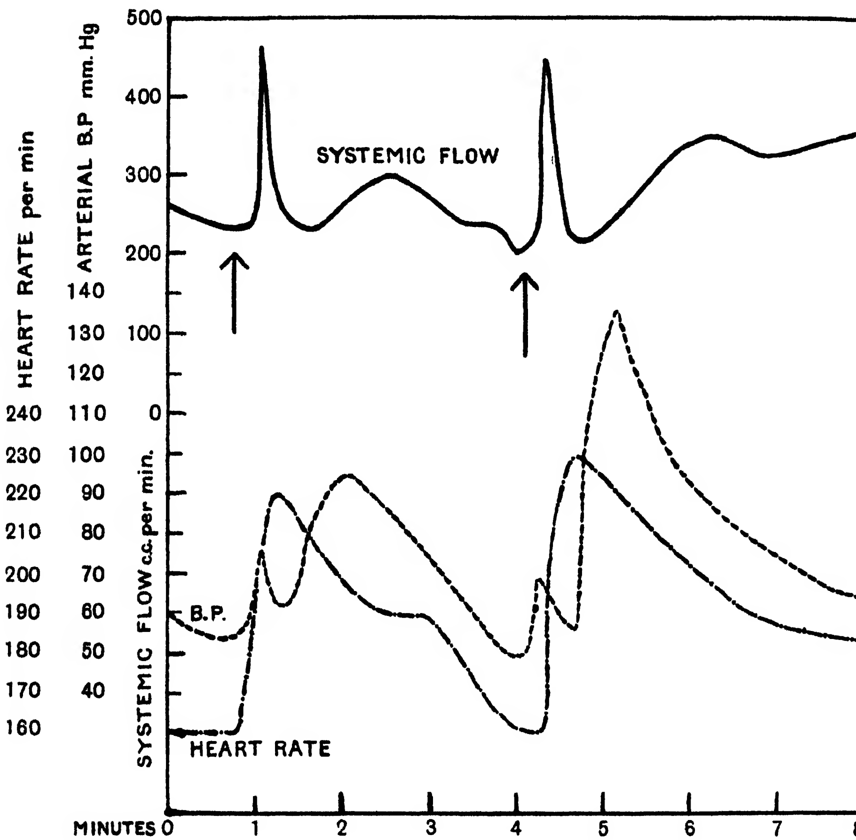

Fig. 2. Dog. Brain destroyed. The arrows mark times at which intravenous injections of adrenaline were made.

(4) The systemic flow is at first decreased by the aortic injections. This decrease accompanies the initial rise in the arterial blood-pressure.

The above effects were found in all typical experiments.

Further experiments of a different nature have provided some explanation for the above changes in the systemic flow.

II. *The influence of adrenaline on the cardio-pulmonary system causing increase in systemic flow.*

To observe how the action of adrenaline upon the cardio-pulmonary system affected the systemic flow, the masking effect of its action on the peripheral vascular system had to be eliminated. This was done by using a preparation in which an artificial peripheral vascular system replaced

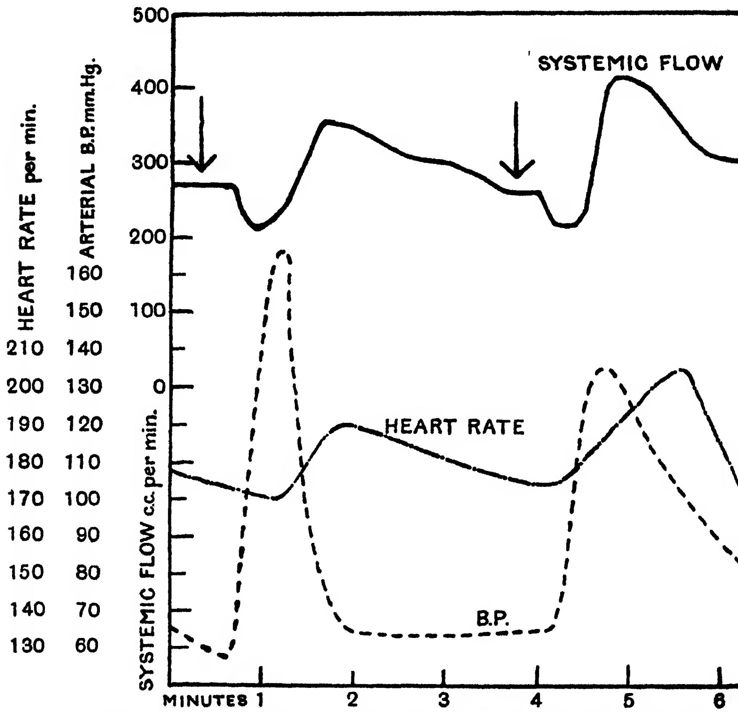

Fig. 3. Dog. Brain destroyed. The arrows mark times at which adrenaline was injected into the aorta.

the animal's peripheral vascular system. The preparation is fully described in a previous paper [Barcroft, 1931 *b*] and is diagrammatically shown in Fig. 1 *b*.

The following typical result has been obtained. Fig. 6 I shows that the flow round the artificial peripheral vascular system was greatly increased by adrenaline.

The conclusion drawn from this experiment is that, in the animal, the action of adrenaline on the cardio-pulmonary system tends to increase the systemic flow in qualitatively the same manner as shown in Fig. 6 I.

III. *The influence of adrenaline upon the peripheral resistance causing decrease in systemic flow.*

The fact that adrenaline causes increase in the arteriole tone and in the peripheral resistance is well known.

To imitate this action a screw clamp was placed in position round the animal's aorta at the point *F* shown in Fig. 1 *a*. Increase in the peripheral

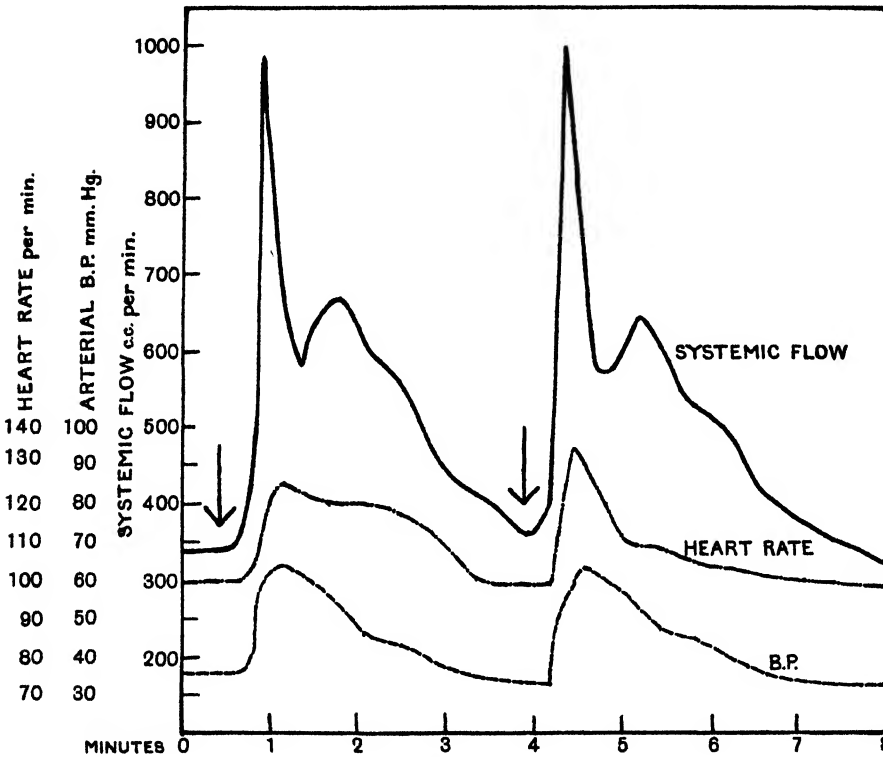

Fig. 4. Dog. Brain destroyed. The arrows mark times at which intravenous injections of adrenaline were made.

resistance was effected by partially tightening the screw, thereby partially occluding the aorta. Typically increase in the peripheral resistance always decreased the systemic flow. Fig. 6 II shows that in one instance increase in the peripheral resistance caused the arterial blood-pressure to rise from 140 to 180 mm. of mercury. The systemic flow dropped from 800 c.c. to 580 c.c. per minute and remained constant till the occlusion was removed 40 seconds later.

The conclusion drawn from this experiment is that, in the animal, the action of adrenaline causing increase in the peripheral resistance tends to decrease the systemic flow.

IV. *Explanation of variations in the animal's systemic flow due to adrenaline.*

In Fig. 7 I, III, the blackened areas have been drawn to represent a hypothetical example of the extent and duration of the increased systemic output caused by the action of adrenaline upon the cardio-pulmonary system.

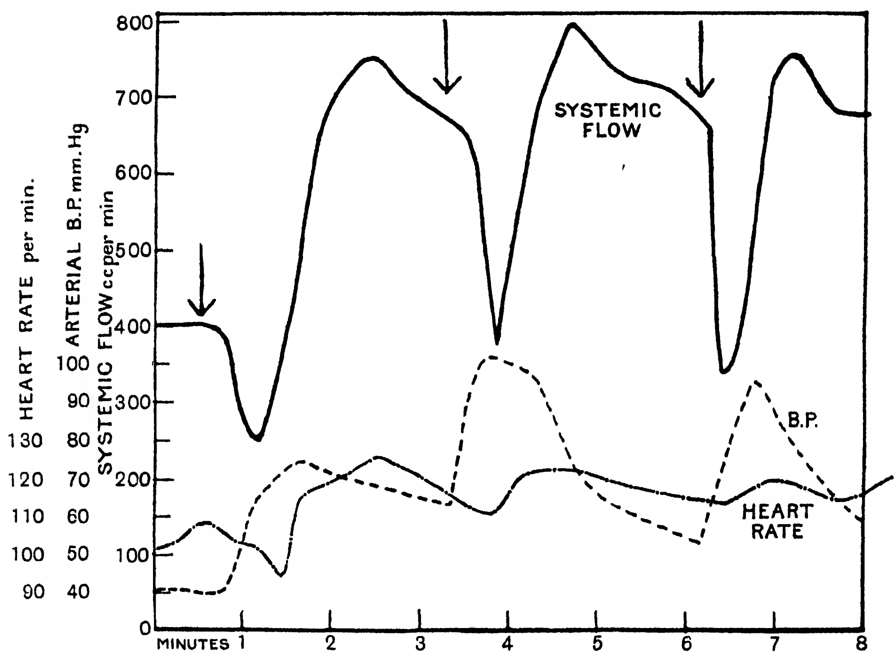

Fig. 5. Dog. Brain destroyed. The arrows mark times at which adrenaline was injected into the aorta.

The shaded areas represent a hypothetical example of the extent and duration of the influence of adrenaline upon the peripheral resistance.

Diagram I is an imaginary illustration of the course of events after an injection of adrenaline into the aorta. The influence of adrenaline upon the peripheral resistance precedes its influence upon the cardio-pulmonary system. In diagram II the algebraic sum of these changes in the flow has been plotted. The shape of the curve is essentially the same as that of the curves obtained for the aortic injections in the animal (Figs. 3, 5).

Diagram III is an imaginary illustration of the course of events after the intravenous injection of adrenaline. The influence of the adrenaline upon the cardio-pulmonary system precedes its influence upon the peri-

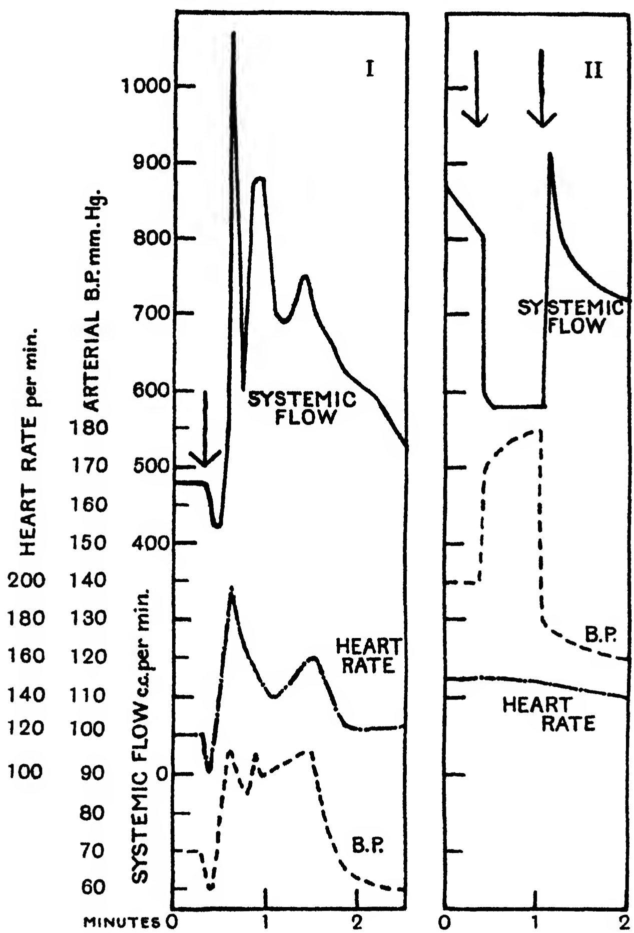

Fig. 6.

I. Preparation embodying artificial peripheral vascular system. The arrow shows time at which adrenaline was injected into the artificial peripheral vascular system.

II. Animal. During the time between the arrows the systemic flow was partially obstructed.

pheral resistance. In diagram IV the algebraic sum of these changes has been plotted. The shape of the curve is essentially the same as the shape of the curves obtained after intravenous injections in the animal (Figs. 2, 4).

Differences in the extent and duration of the influence of adrenaline

upon the peripheral resistance and upon the cardio-pulmonary system in different animals appear to be responsible for determining the extent to which the output is affected. Further experiment may establish additional factors by which adrenaline can influence the systemic output, and may lead to an amplification of the present explanation of its action.

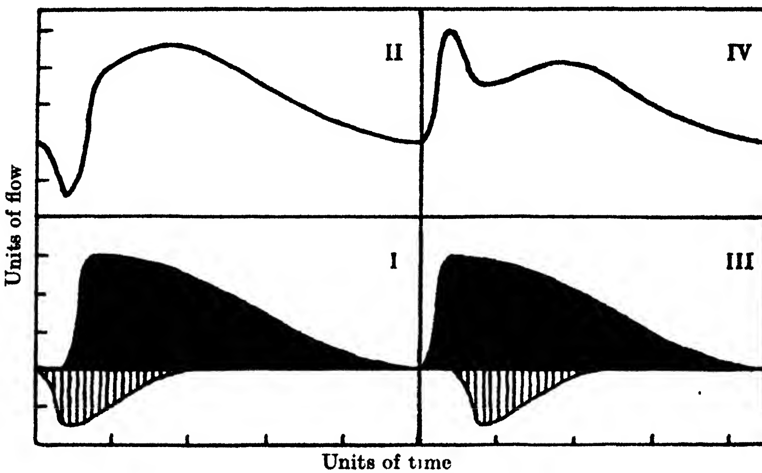

Fig. 7.

SUMMARY.

1. The influence of adrenaline upon the systemic output has been studied in dogs. The mechanical stromuhr was used to record the systemic flow.

2. In some animals the systemic output is not much increased or decreased, in others it is considerably increased.

3. Evidence is brought forward to show that the influence of adrenaline upon the peripheral resistance tends to decrease the systemic flow.

4. Evidence is brought forward to show that the influence of adrenaline upon the cardio-pulmonary system tends to increase the systemic flow.

5. The variable results of adrenaline injections appear to be due to variations in the extent of its influence upon the peripheral resistance and upon the cardio-pulmonary system.

I am very grateful to Prof. G. V. Anrep for suggesting the subject of this research.

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612.215.3:547.781.5

THE ACTION OF HISTAMINE ON THE RESPIRATORY TRACT.

By DAVID EPSTEIN.

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DALE and LAIDLAW [1910] have shown that injections of histamine produce effects which closely resemble those seen in anaphylactic shock and asthmatic attacks. One of the chief symptoms is obstruction of the respiratory passages, and this is usually attributed to constriction of the bronchi or bronchioles. The obstruction occurs in varying degree in most animals, and in the guinea-pig it is so marked a feature as to cause death from asphyxia, the lungs at post-mortem being usually found greatly distended.

While working in the laboratory of Prof. J. A. Gunn at Oxford the author [Epstein, Gunn and Virden, 1932] evolved a modification of Trendelenburg's method of recording isolated rings of bronchi [Trendelenburg, 1912]. These preparations responded to most autonomic drugs in the same way as the plain muscle of the intestine. Histamine, however, failed to cause constriction of the bronchial rings of the cat and under certain conditions actually produced relaxation.

In view of this apparently conflicting evidence of the action of histamine on the bronchi it was decided to carry out a detailed investigation into the action of the drug on the respiratory passages. Since the responses to peptone more closely resemble the symptoms of anaphylactic shock and asthma than those produced by histamine, some experiments have also been performed with this drug. A preparation of peptone manufactured by Morson and Son was employed. Most of the work has been carried out on cats and guinea-pigs, but a few experiments have also been done on rabbits. Freshly prepared solutions of ergamine acid phosphate (B.W. and Co.) were used in the experiments and the doses refer to this salt, *e.g.* "1 mg. of histamine" means 1 mg. of this preparation.

Experimental methods and results.

I. *Lung volume records.*

The lung volume changes in the cat were recorded by means of sternal- and lung-shields as described by Jackson [1913, 1914, 1917]. Voluntary respiration was abolished by destroying the medulla by plunging a sharp instrument through the atlanto-occipital membrane and plugging the wound immediately afterwards with cotton-wool. (Previously both vagi had been cut and both carotid arteries ligatured under ether.) Artificial respiration was immediately started.

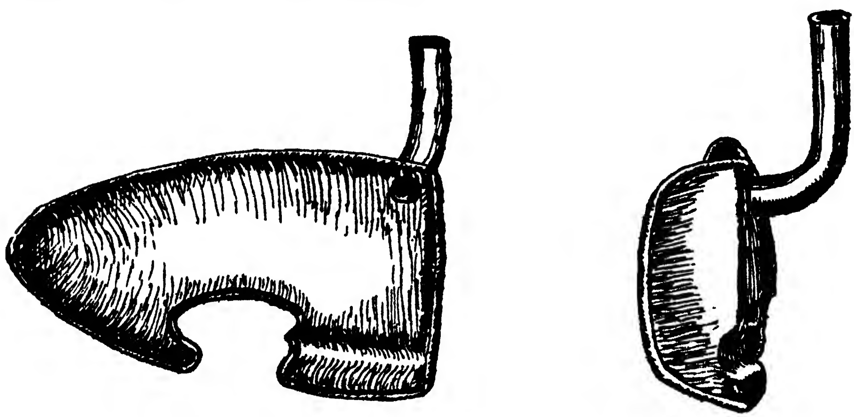

Fig. 1. Lung-shield (after Jackson) with a metal side tube for connecting to a tambour. (See text.)

The sternal-shield method of registering the lung volume changes requires very little manipulation, but unfortunately the records are complicated by the volume changes of the heart. The lung-shield method, however, gives good results provided it is carefully placed in position so as not to damage or compress the vessels at the root of the lung. When the shield is in position Jackson records the lung changes by making another opening in the chest wall, through which is passed a tube connected to a tambour. The latter step was obviated by soldering a hollow metal tube to the shield at the junction of the mediastinal and diaphragmatic surfaces close to the anterior border (Fig. 1). The tube (bent at right angles) projected through the original incision in the chest wall and was connected by a rubber tube to a tambour. The metal tube was clamped to a stand to keep the shield in the desired position. Positive pump pressure

was used to inflate the lungs, the pump having been connected to a cannula in the trachea.

Intravenous injections of histamine caused a definite diminution in the lung volume with doses of 0·1 mg. per kg. But even with much larger doses it was often impossible to cause complete occlusion. For example, in Fig. 2 a dose of 5 mg. per kg. of histamine caused a marked but incomplete obstruction which was sufficient, however, to bring on symptoms of

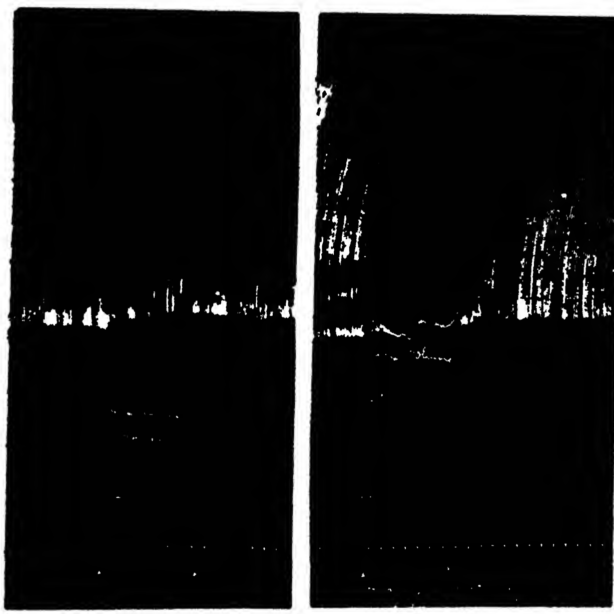

Fig. 2. Cat. Brain pithed. Lung volume. Shows marked but incomplete obstruction of air passages after 5 mg. per kg. histamine. Arecoline (0·25 mg. per kg.) produced complete air obstruction, which was partially abolished by atropine.

asphyxia, a fatal termination being prevented by mechanically dilating the bronchi. In contrast with these results complete diminution of volume could easily be produced by small injections of arecoline (Fig. 2).

Changes in lung volume similar to those in the cat were produced in pithed rabbits. It was found that the blood-pressure of these animals, however, was not well maintained.

Guinea-pigs were given doses of paraldehyde (2 c.c. or more per kg.) intraperitoneally in order to paralyse the respiratory centre. The lung volume was recorded by simply making a small incision in the chest wall

so that a thistle funnel could be squeezed in. The aperture was then rendered airtight and the tube connected to a tambour. The injections of histamine produced powerful effects, even such small doses as 0·01 mg. per kg. sometimes causing complete obstruction to the exchange of air in the lungs (Fig. 3). In one experiment, in which voluntary respiration had been abolished by paraldehyde, histamine caused the onset of inspiratory movements, as if the drug had stimulated the respiration.

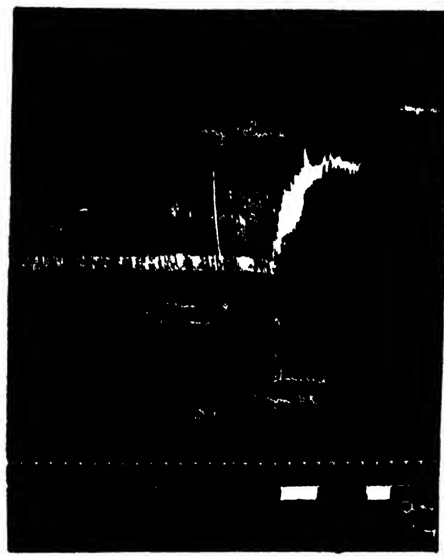

Fig. 3. Guinea-pig. Respiratory centre paralysed by paraldehyde. Shows complete air obstruction after 0·1 mg. per kg. histamine. Repeated injections of adrenaline failed to relieve the effect and animal died of asphyxia.

From the experiments described above it is seen that histamine causes obstruction to the passage of air into or out of the lungs of the cat, rabbit and guinea-pig. The guinea-pig is much more susceptible than the cat or rabbit, small doses sufficing to cause complete obstruction. In the cat large doses also cause a certain degree of occlusion, but even very large doses often fail to produce complete obstruction. These results are in close agreement with the findings of Dale and Laidlaw [1910]. Similar effects were seen with peptone, *e.g.* 0·1 gm. of peptone per kg. also produced partial occlusion of the air passages of a cat.

The obstruction in the respiratory tract described above is usually attributed to constriction of the bronchi or bronchioles. The lung volume

records, as obtained above, however, are complicated by circulatory changes in the lungs under observation, and these pulmonary vascular changes may be important factors in causing obstruction in the respiratory passages. In order to determine the relative importance of (1) the vascular changes in the lung, and (2) the direct action of the drugs on the bronchi, in causing obstruction in the respiratory passages, further experiments were carried out.

II. *Isolated rings of trachea or bronchi.*

Trendelenburg [1912] introduced the method of investigating the direct reactions of the bronchi by means of the isolated bronchial ring preparation. He employed the bronchi of the ox, carefully dissecting away the cartilage and suspending the remaining plain muscle in a bath of Ringer's solution warmed to body temperature. The lever had to be weighted fairly heavily to remove the marked degree of tone usually present in the muscle. Macht and Ting [1921] carried out a series of investigations on the bronchi of the pig, following the procedure of Trendelenburg very closely. Titone [1913] investigated the isolated bronchi of the calf, sheep, pig and dog, merely suspending the rings without previously dissecting away the cartilage as described by Trendelenburg. The excessive tone, however, had again to be abolished by weighting. Golla and Symes [1913] investigated an isolated muscle strip of the cat's trachea, although they showed no tracings of these experiments. Franklin [1926] used an isolated ring of a sheep.

In the present investigation a number of isolated rings of the trachea or bronchi have been strung together side by side like the wheels of a tandem bicycle. According to the diameter of the rings it has been usual to employ from 7 to 14 rings, which together with a lever magnification of 12 to 16 times has magnified the reactions of each ring approximately 100 to 200 times. Using this method the author has been able to record isolated rings of the trachea or bronchi of the cat, rabbit and guinea-pig.

After the animal had been killed by a blow on the back of the neck and bled by severing the vessels in the neck, the trachea was carefully dissected out and cut into sections to give rings of various widths. In the cat the diameter of the trachea was usually 5 to 10 mm., and fairly wide rings were cut, *e.g.* about 6 mm. In the case of the guinea-pig, however, the diameter of the trachea was very much smaller (about 3 to 4 mm.), and so rings having a width of about 3 to 4 mm. were used. Also, the bronchi as they passed into the lungs were carefully dissected out. These tubes which had an average diameter of about 1·5 mm. were carefully

cleaned of all lung tissue, cut into sections of about 3 mm. wide and then strung together tandem fashion, using a needle and thread.

When the rings of trachea or bronchi were tied together the string was made to encircle the cartilaginous portions of the rings, when possible, so as to avoid tearing the intercartilaginous strips of plain muscle.

The preparations were then suspended in a bath of Ringer's solution maintained at 37° C., one end being fixed to a glass rod and the other end attached to a lever by means of a thread. Air was bubbled through the Ringer's solution (NaCl 0·9 p.c., KCl 0·042 p.c., $CaCl_2$ 0·022 p.c., and $NaHCO_3$ 0·02 p.c.) at a constant rate. In contrast with the methods of Trendelenburg and Macht, in which heavy weighting of the lever was necessary to overcome the excessive tone of the preparations, in the present method very lightly balanced levers were required. The rings have never shown spontaneous movements when set up in Ringer's solution, but the addition of certain drugs has sometimes produced irregular rhythmic movements. These effects, together with the responses of the rings to other drugs, will be described in another paper.

It was found that the ring preparations were normally fully relaxed, so that the actions of broncho-constrictor drugs could be studied by merely adding the drugs to the baths containing the preparations. Constriction of the rings had first to be produced, however, before any effect could usually be obtained with broncho-dilator drugs. To obtain the preliminary constriction in these cases it was found that arecoline and acetyl choline were both suitable, because their dosage could be graded to give effects which were quite definite but which would not mask the relaxing effects of broncho-dilator substances when given subsequently in sufficient concentration. When at rest the preparation usually gave horizontal straight lines, so that the action of constrictor drugs could be easily seen. When the effects of various drugs had been produced the record again usually consisted of a horizontal line, so that the actions of additional substances were again fairly obvious.

(*a*) Using ring preparations of the cat's trachea or intrapulmonary bronchi it was found that constriction was produced by the parasympathetic group of drugs, and relaxation by the sympathetic group [Epstein, Gunn and Virden, 1932]. Histamine acid phosphate, however, failed to constrict the rings when given in concentrations ranging from 1 in 1,000,000 to 1 in 13,000. In Fig. 4 the concentration has gradually been increased, but each concentration has been tried on previously untreated preparations in other experiments (Fig. 5).

In the course of the investigation it was found that after histamine

arecoline often failed to constrict the rings in concentrations which had been active when given previous to the histamine (Fig. 5). The action of

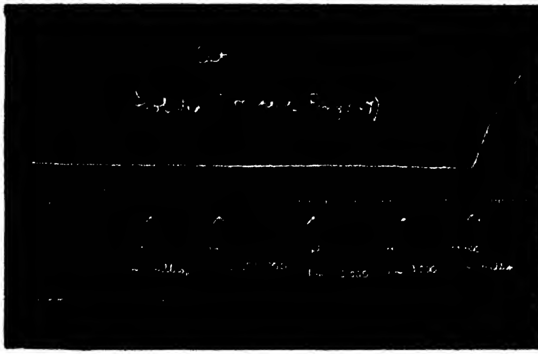

Fig. 4. Reduced to $\frac{2}{3}$ of original size. Cat. Isolated tracheal rings (7). Concentrations of histamine (1 in 1 million; 1 in 300,000; 1 in 100,000 and 1 in 13,000) all had no effect on the fully relaxed preparation. Arecoline (1 in 1 million) however produced a powerful contraction.

Fig. 5. Reduced to $\frac{1}{2}$. Cat. Isolated tracheal rings (8). Histamine (1 in 100,000) had no apparent effect on the fully relaxed preparation. Arecoline in a concentration of 1 in 8 million failed to have any effect in the presence of histamine (compare with the effect shown later in the figure when histamine has been washed out); but increasing the concentration to 1 in 2 million caused a powerful contraction. Replacing the solution with Locke's solution (twice) caused the rings to relax gradually. The rings were now constricted by arecoline in concentrations of 1 in 20 million and 1 in 10 million. Histamine (1 in 100,000) produced a marked relaxation of the contracted rings, and adrenaline (1 in 3 million) caused a further relaxation.

histamine was therefore tested after the constriction produced by weak concentrations of arecoline or acetyl choline. Under these conditions histamine often produced relaxation of the cat's tracheal or bronchial

rings (Figs. 5 and 6). The arecoline and acetyl choline contraction was only partially abolished by histamine, and stronger concentrations of arecoline or acetyl choline were able to cause a further degree of constriction. It may be claimed that the relaxation seen with histamine is

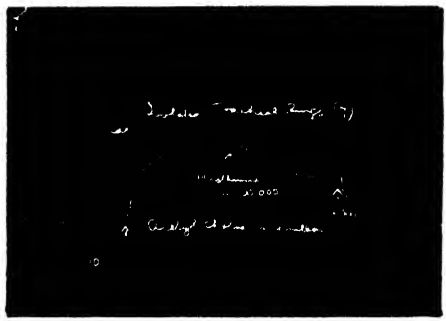

Fig. 6. Reduced to ½. Cat. Tracheal rings (7). Contraction with acetyl choline (1 in 12 million) and relaxation with histamine (1 in 100,000). Further relaxation on replacing solution with Ringer's fluid.

Fig. 7. Reduced to ½. Cat. Intrapulmonary bronchi (12 rings). Histamine in a strength of 1 in 1 million had no effect; 1 in 100,000 produced a slight relaxation; 1 in 15,000 had no effect. Arecoline (1 in 400,000) caused a contraction, which gradually passed off when replaced by fresh Ringer's solution (L.S. 2). Time in 10 seconds. The time signal was lowered at X.

due to the drug having a weak atropine-like effect and being able in certain proportions to abolish the effects of parasympathetic drugs like arecoline. However, in two experiments when the untreated rings were apparently not fully relaxed and still retained a slight degree of tone, concentrations of histamine of 1 in 100,000 or stronger produced a slight relaxation (Fig. 7). Further experiments were also carried out with

barium chloride. The constriction produced by barium was usually complicated by the occurrence of irregular rhythmic waves, so that the effects of subsequent drugs were sometimes difficult to interpret. It was found, however, that if given in strong concentrations, *e.g.* 1 in 10,000, histamine

Fig. 8. Same experiment as in Fig. 7. Peptone in concentrations of 1 in 1 million; 1 in 100,000 and 1 in 20,000 had no effect on the fully relaxed rings. Arecoline (1 in 400,000) caused contraction, adrenaline (1 in 500,000) produced relaxation and atropine (1 in 1 million) produced a further lowering of tone to normal.

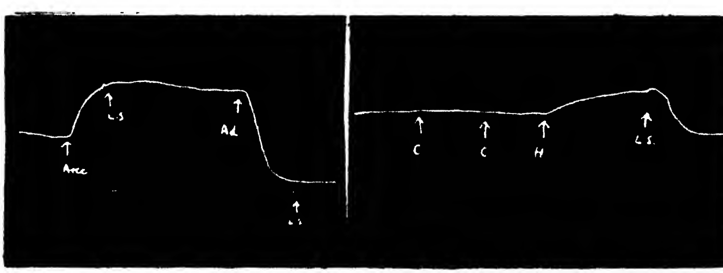

Fig. 9. Reduced to ½. Guinea-pig. Isolated tracheal rings (8). Arecoline (1 in 1 million) caused a contraction, which was unaffected by washing out with Ringer's solution (L.S.). Adrenaline (1 in 2 million) produced relaxation. Drugs washed out and replaced by fresh Ringer (L.S.). Lever raised. At *C* 0·5 c.c. of Ringer's solution was added to the bath. This was repeated but no effect was produced in either case. These served as controls. At *H* histamine was added to give a concentration of 1 in 300,000. This caused constriction of the rings, which disappeared on washing out with fresh Ringer (L.S.).

sometimes produced a weak relaxation after barium, or abolished the rhythmic waves. Under these conditions the strong concentrations of histamine occasionally gave a preliminary transient weak contraction before showing the slight degree of relaxation.

Peptone in concentrations of 1 in 1 million to 1 in 20,000 also failed to contract the cat's bronchi (Fig. 8), and the strong concentrations relaxed the tissue if previously constricted with arecoline.

(*b*) With the tracheal rings of the rabbit histamine again failed to cause constriction when given in concentrations of 1 in 4 million to 1 in 200,000. Histamine occasionally produced relaxation after the contraction caused by other drugs.

(*c*) In contrast with the results in the cat and rabbit it was found that the tracheal rings of the guinea-pig were constricted by histamine. Concentrations of 1 in 1,000,000 to 1 in 75,000 were used (Figs. 9 and 10). In one experiment performed on rings of the bronchi the effects were poor, but a weak constriction was again obtained with histamine.

In connection with these results it is of interest to note that Trendelenburg [1912] failed to obtain any effect with histamine on the isolated bronchi of the ox, Macht and Ting [1921] described contraction of the isolated bronchi of the pig, Titone [1913], working with bronchial rings of the calf, sheep, pig and dog, also recorded concentrations with histamine, but Golla and Symes [1913] obtained no effect on the trachea of the cat.

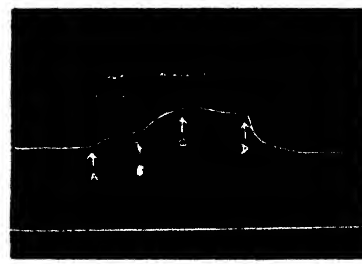

Fig. 10. Reduced to ½. Guinea-pig. Tracheal rings (7). At *A* histamine (1 in 1 million) caused constriction. At *B* increasing the concentration to 1 in 300,000 produced a further constriction. At *C* tyramine acid phosphate (1 in 100,000) caused an insignificant degree of relaxation. At *D* adrenaline (1 in 1 million) caused complete relaxation.

(i) From the results obtained above it would appear that the obstruction of the respiratory passages, that occurs in the guinea-pig after histamine, is due to a direct stimulant action of the drug on the musculature of the respiratory tract. As shown above, constriction of the trachea and bronchi appears to take a definite part in this action, and probably constriction of the bronchioles also occurs.

(ii) On the other hand, although it has been shown that histamine also causes obstruction of the respiratory passages of the cat [Dale and Laidlaw, 1910, and others—see also Section I], the experiments described above indicate that this drug does not cause contraction of the trachea and intrapulmonary bronchi but may actually relax these structures. The obstruction of the respiratory tract in the cat with histamine must therefore be due to a direct constriction of the bronchioles by the drug, or it is possible that the changes in pulmonary circulation that occur with histamine cause a mechanical compression of the respiratory tubes. In order to decide these points some experiments were performed with histamine on the pulmonary circulation of the cat.

III. *Pulmonary circulation.*

Dale and Laidlaw [1910] showed that histamine caused a fall in the systemic blood-pressure in the cat, but produced a marked rise in the pulmonary blood-pressure, the latter effect being attributed to constriction of the pulmonary arterioles. Dixon and Hoyle [1930] claimed that histamine produced "congestion of the whole pulmonary circulation," for they found that there was an increase in the blood volume in the lung and an increased blood flow through the lung, these changes leading to a rise in pulmonary pressure.

Other investigators have shown that vascular congestion of the lungs produces obstruction of the respiratory passages. Drinker and Agassiz [1925] produced congestion of the pulmonary circulation by clamping the pulmonary veins of the cat. This caused a reduction in the amount of air that could be pumped into the lung, but the condition returned to normal again when the vein clamp was removed, showing that the changes had not been due to the formation of œdema in the lungs. They concluded that the obstruction to air entrance produced by pulmonary vascular congestion was "feeble in the normal non-œdematous and non-fibrotic lung tissue of the cat." By taking measurements on some of the graphs in this paper the author found that the reduction in the amount of air passing into the lungs never exceeded 25 p.c. of the total even during the severest degrees of pulmonary congestion.

Hanzlik [1925] clamped the pulmonary veins of the guinea-pig and found that the cast obtained by pouring celloidin into the trachea and bronchi of the excised lungs was markedly reduced, indicating that obstruction of the respiratory passages had occurred, presumably by vascular compression of the bronchioles.

To determine the part played by the pulmonary vascular congestion seen with histamine in causing obstruction of the air passages, simultaneous records of the pulmonary blood-pressure and the lung volume were taken in cats. The animals were anæsthetized with intraperitoneal injections of paraldehyde, a dose sufficient to paralyse the respiratory centre (usually about 2 to 2·5 c.c. per kg.) being used. Artificial respiration was carried on by a pump attached to the trachea, positive pressure being again employed. The volume of one of the lower lobes of the right lung was recorded by cutting away the ribs on the right side of the chest and enclosing the lobe in an oncometer after the manner of Dixon and Brodie [1903]. The oncometer used in the present experiments consisted merely of a wide glass cylinder with a narrow, oval-shaped orifice at one

end, the other end being tightly corked; a tube passing through the cork connected the apparatus with a tambour. As shown by Dixon and Brodie, handling of the lung produces a collapsed organ which fails to distend adequately under pump pressure. It was found that by placing the oval-shaped orifice of the oncometer in contact with the lung and applying suction at the other end of the apparatus, the lung could be sucked into position in the oncometer with the least possible damage. The oval-shaped orifice was then rendered airtight by blocking it with vaseline or by clamping the apparatus at an angle fairly tightly into the chest cavity. The pulmonary blood-pressure was recorded at the same time by placing a cannula into the branch of the pulmonary artery supplying the middle lobe of the left lung, half-saturated sodium sulphate being used in the manometer. The systemic blood-pressure was recorded by means of a cannula in the left carotid artery and the drugs were injected slowly into the right femoral vein.

Fig. 11 illustrates the results of such an experiment. The injection of 0·2 mg. per kg. of histamine caused a marked rise in pulmonary blood-pressure (75 mm.) and a fall in systemic blood-pressure. The lung volume was at the same time diminished, indicating that marked obstruction had occurred in the respiratory passages. The respiratory obstruction commenced about 10 seconds after the beginning of the rise in pulmonary blood-pressure and both reached a maximum about the same time. The pulmonary pressure returned to normal after 78 seconds, but at this time marked obstruction of the air passages was still present, the record showing a 50 p.c. reduction in the amount of air that could be pumped into the lung. The lung volume returned to normal only after 4 minutes. These results show that the obstruction of the air passages produced by histamine can persist long after the congestion of the pulmonary circulation has passed off.

It was also found that after repeated injections of histamine both the systemic and pulmonary blood-pressures usually gave very feeble responses with the drug, but that marked obstruction of the air passages could still be produced at each injection of the substance (Fig. 12). These results indicate that histamine is able to cause obstruction of the air passages in the cat quite independently of any changes produced in the pulmonary circulation.

There was still the possibility that the pulmonary vascular congestion served as an accessory factor in producing the air obstruction seen with histamine. However, it was noted that the degree of air obstruction produced by the same doses of histamine was the same, whether there had

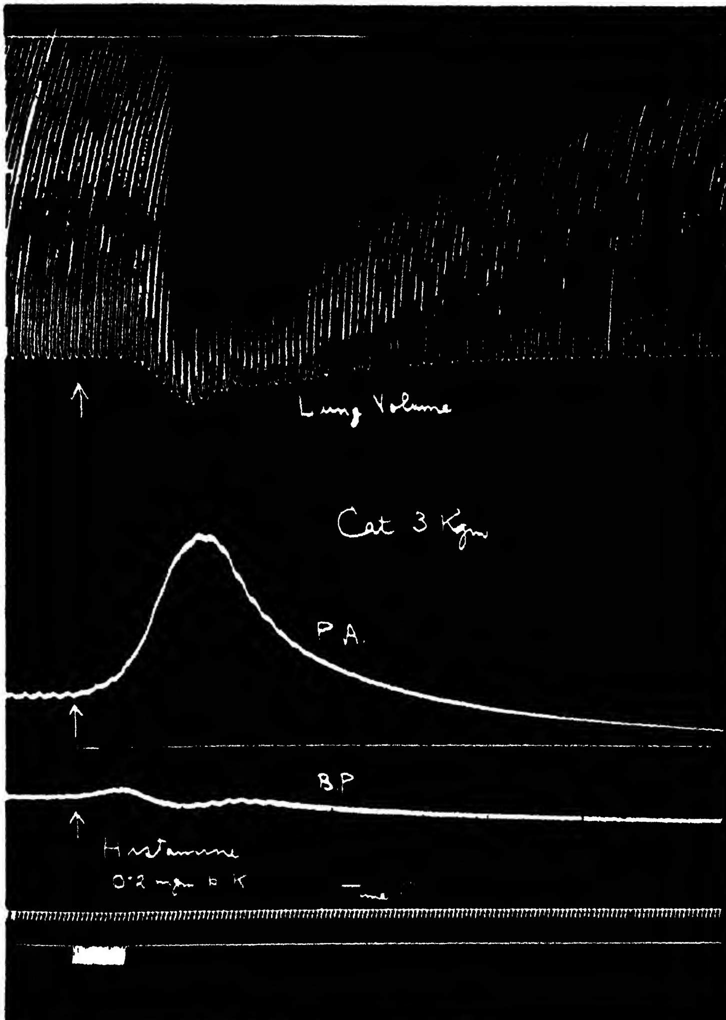

Fig. 11. Cat. The injection of 0·2 mg. per kg. of histamine produced a diminution of lung volume (upper record), a rise in pulmonary pressure (middle record) and a fall in systemic pressure (lower record). (See text.) Time in seconds.

been a marked rise in pulmonary pressure or whether (later on in the same experiment) the change in pulmonary pressure had been negligible. Thus in Fig. 12 an injection of 0·1 mg. per kg. of histamine produced an air obstruction of 35 p.c. although the rise in pulmonary pressure was only 5 mm.; while the same dose of histamine given earlier in this experiment produced the same degree of air obstruction but the rise in pulmonary pressure was 25 mm. Again, in another experiment an injection of 0·05 mg. per kg. of histamine caused a rise in pulmonary pressure of 35 mm. of half-saturated sodium sulphate solution, and there was a reduction in the passage of air of only 7 p.c. Later on in the experiment the same degree of air obstruction occurred with this dose of histamine, but there was a rise in pulmonary pressure of only 8 mm. These experiments suggest that any accessory value that the pulmonary vascular changes may have in increasing the air obstruction seen with histamine is probably insignificant.

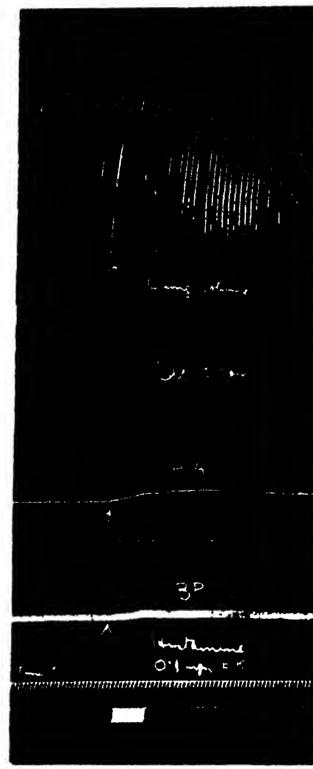

Fig. 12. Same experiment as in Fig. 11 after repeated injections of histamine. Shows that a further injection of histamine (0·1 mg. per kg.) has a negligible effect on the pulmonary blood-pressure, but is still able to produce a diminution of lung volume.

Dale and Laidlaw [1910] claim that histamine increases the bronchial secretion and this might tend to cause some obstruction of the respiratory tract, which would persist until the passages had again been cleared. In many of the experiments, however, the lung volume records showed that the obstruction of the air passages passed off completely if the doses of histamine had been small and had not been repeated too frequently, indicating that any increase in bronchial secretion played a relatively unimportant part in causing respiratory obstruction.

Sometimes with large frequently repeated doses, however, the obstruction passed off only partially, even if atropine had been previously given to prevent any possible increase in bronchial secretion. The persistence of the obstruction in these cases may perhaps be partly explained by assuming that a certain amount of œdema of the lungs had occurred

as a result of the repeated pulmonary vascular congestion, and that this was causing a partial mechanical obstruction of the respiratory passages. It is also possible that an angioneurotic œdema of the respiratory mucous membrane, and especially of that portion lining the bronchioles, may be the cause of air obstruction seen with histamine. However, if such were the case, one would expect the obstruction to persist for some considerable time, whereas it has been shown that with small doses of histamine the condition often passes off completely fairly soon. In the more persistent air obstruction seen after large frequently repeated doses of histamine it is possible that this condition may be present and serve as an accessory factor.

From the experiments described in this section it has been shown that histamine can cause obstruction of the respiratory passages of the cat quite independently of any changes in the pulmonary circulation and of changes in the amount of bronchial secretion. While the accessory value of the pulmonary vascular congestion in increasing the obstruction is insignificant, it is possible that this congestion when frequently produced may in certain cases be followed by some pulmonary œdema, which may mechanically impede to some extent the passage of air through the respiratory passages. It is also possible that increased bronchial secretion produced by histamine may in certain cases serve mechanically as an accessory factor in increasing the air obstruction.

IV. *Perfusion of the lungs through the respiratory passages.*

Baehr and Pick [1913] and McDowall and Thornton [1930] have investigated the responses of bronchi by perfusing drugs through the pulmonary vessels. To eliminate any possible vascular effects a method of direct perfusion of the drugs through the respiratory passages, based on the method described by Sollmann and von Oettingen [1928], was employed in the present investigation.

The animals were killed by a blow on the back of the neck and bled by severing the vessels in the neck. The lungs and trachea were excised intact, placed in a large dish of Ringer's solution and gently massaged until as much air as possible had been squeezed out. Both lungs of the guinea-pig were perfused by tying a cannula into the trachea just above its bifurcation; in cats only one lung was perfused, the cannula being tied into the main bronchus. The preparations were now connected to the perfusing apparatus (Fig. 13) and sufficient Ringer's fluid allowed to flow in to distend the lungs slightly. The lungs were massaged again to squeeze out any remaining air, the bubbles being allowed to escape through a side

tube just above the cannula. The side tube was then closed and sufficient Ringer's solution allowed to run in to distend the lungs moderately. A number of shallow punctures was made with a needle through the surface of the lungs, especially in the neighbourhood of the borders of the lungs. The fluid was thus able to pass through the trachea, bronchi and bronchioles, and escape through the punctured alveoli. The height of the Mariotte bottle was arranged so that the pressure of fluid was just sufficient to distend the lungs to about their normal size. In the case of the

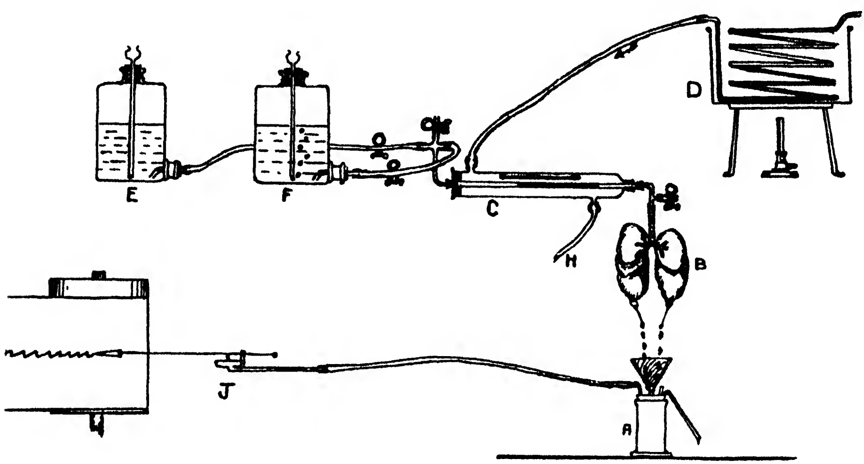

Fig. 13. Showing arrangement of apparatus used for perfusing the respiratory passages. A = Gunn's siphon-recorder; B = lungs with threads attached so that fluid collects in funnel; C = Gunn's warming jacket; E = Mariotte bottle containing Ringer's solution; and F = bottle with a definite concentration of drug to be investigated. The rubber tubes connecting E and F to C should be of equal length and should be relatively shorter than shown. The heights of the bottles should be as indicated in the text.

cat it was found that the lower end of the tube in the Mariotte bottle should be about the same level as the tip of the cannula in the bronchus. The lowest point of the dependent lung was usually about 6 cm. below the cannula tip, so that the perfusion height in these cases was about 6 cm. Under these conditions excellent results were obtained, but if the perfusion pressure was increased the lungs became over-distended and even strong concentrations of arecoline, etc., had no appreciable effect. In guinea-pigs, however, it was found that greater perfusion heights could be used, *e.g.* good results were obtained with a total height of 15 cm. Sollmann and von Oettingen measured the inflow by counting or recording the air bubbles entering the Mariotte bottle. In the present

experiments it was found more convenient to measure the outflow, and typical results could be obtained even after the preparation had been up for 1½ to 2 hours. The outflow was recorded by Gunn's siphonometer [Gunn, 1913 *b*]. To collect the fluid from such a large surface as the cat's lung a wide funnel could be used on the siphon-recorder or by attaching threads to the projecting lowest points of the lung the fluid could be made to collect in the mouth of the funnel. The upper lobe of the cat's lung usually failed to distend with fluid, probably because the cannula had been tied in beyond the orifice of the bronchus to this lobe. To eliminate any possibility of fluid occasionally being able to enter this lobe and so produce erroneous results, the lobe was tied off at the beginning of the experiment. The perfusing fluid was warmed to 37° C. by means of Gunn's apparatus for perfusing the isolated mammalian heart [Gunn, 1930 *a*]. Since the perfusion height required was not very great, it was found more convenient to have the warming jacket in the horizontal plane (or with a very slight incline) than in the vertical position. This eliminated having to use long portions of rubber tubing connecting the Mariotte bottles to the warming jacket, and when changing perfusing fluids the drugs in the Mariotte bottles were able to reach the lung very quickly. When the outflow was constant under perfusion with Ringer's fluid, a solution of the drug to be investigated was allowed to flow in from another Mariotte bottle, keeping the perfusion pressure unaltered. In these experiments the exact strength of the drug employed was known. In other experiments 1 c.c. of a concentrated solution of the drug was injected into the rubber tube just above the lung cannula.

Histamine produced marked diminution in the outflow from the lungs of both animals, but the effects in the guinea-pig were many times more powerful than those in the cat. A concentration of 1 in 5 million histamine caused a definite obstruction to the flow of fluid through the trachea and lungs of the guinea-pig. A strength of 1 in 500,000 produced almost complete obstruction, the outflow being reduced from 18 c.c. per minute to 0·4 c.c. per minute (Fig. 14, lower record). In the upper record of the same figure an injection of 1 c.c. of a 1 in 2000 solution of histamine into the perfusing fluid reduced the outflow from 45 c.c. to 5 c.c. per minute, adrenaline (1 c.c. of 1 in 1000) causing a great increase again.

Weak effects were seen with solutions of 1 in 500,000 histamine in the cat's lung, but with 1 in 100,000 marked obstruction occurred, the outflow being reduced from 9 c.c. to 1 c.c. per minute (Fig. 15, upper record), but this was increased again by an injection of 1·5 c.c. of adrenaline (1 in 1000) and by later changing over to Ringer's solution. An injection of

1 c.c. of a 1 in 1000 solution of histamine reduced the outflow from 13 c.c. to 2 c.c. per minute (lower record). Weak effects were also seen with peptone.

Upper record
Lower record

Fig. 14. Reduced to ½. Guinea-pig. Lung perfusion record. Each notch represents an outflow of 3 c.c. Time record shows intervals of 10 seconds. Lower record: at the arrow perfusion was changed from Ringer's solution to a 1 in 500,000 solution of histamine, which resulted in almost complete obstruction to the outflow. Upper record: the injection of 1 c.c. of a 1 in 2000 solution of histamine into the rubber tube just above the tracheal cannula caused a marked diminution in outflow. The injection of 1 c.c. of adrenaline (1 in 1000) greatly increased the outflow.

Upper record
Lower record

Fig. 15. Reduced 70 p.c. Cat. Upper record: at the first arrow the perfusing fluid was changed over from Ringer's solution to a 1 in 100,000 solution of histamine, and a marked diminution in outflow occurred. At the second arrow an injection of 1·5 c.c. of adrenaline (1 in 1000) was given, which increased the outflow even though the histamine solution was still being perfused. At the next arrow (L.S.) the perfusion was switched back to Ringer's solution. The lower record shows the effect of injecting 1 c.c. of a 1 in 1000 histamine solution and then the result of giving 1 c.c. of 1 in 2000 adrenaline.

These experiments show that histamine can cause obstruction of the respiratory passages of the cat and guinea-pig by a direct action on the respiratory tract, and the effect can thus occur independently of changes produced in the circulation and of other factors. This confirms the findings of Section III.

Discussion.

(*a*) *Guinea-pig.* By means of lung-volume records it has been confirmed that histamine is able to produce obstruction of the respiratory passages of the guinea-pig. By perfusing histamine solutions through the respiratory tract of excised lungs any effects on the pulmonary circulation were eliminated, and the fact that the obstruction was still produced under these conditions indicated that the drug must have a direct action on some portion or portions of the respiratory tract. Using ring preparations of the isolated trachea and bronchi it has been shown that histamine is able to constrict these structures to a marked degree. The air obstruction seen after injections of histamine in the guinea-pig can thus be explained wholly or chiefly by the direct constrictor effect of the drug on the plain muscle of the trachea, bronchi and probably the bronchioles too, *i.e.* by contraction of the musculature of the whole respiratory tract, or at least, the greater portion of it. Schultz and Jordan [1911] have shown that the mucous membrane of the secondary bronchi of the guinea-pig normally projects into the lumen in the form of folds, so that even a slight narrowing of the lumen will suffice to occlude these bronchi completely. This factor, taken in conjunction with the findings in this paper, viz. that the greater portion of the respiratory tract is contracted with histamine, would serve to explain the severity of the obstruction seen in guinea-pigs. The occlusion of the respiratory tract is often so marked that the condition frequently terminates in death from asphyxia.

(*b*) *Cat.* Using lung-volume records it has been confirmed that histamine is able to cause obstruction of the respiratory tract of the cat. By recording at the same time the pulmonary blood-pressure it has been shown that the air obstruction seen with histamine can occur quite independently of any changes in the pulmonary circulation. Any accessory value of the pulmonary congestion in increasing the air obstruction is probably unimportant. The obstruction can still occur, even if an increase in bronchial secretion is prevented, and also in the absence of pulmonary œdema. The obstruction would therefore appear to be due to a direct action of the drug on some portion of the musculature of the respiratory tract. This has been confirmed by directly perfusing the drug through the bronchial tree, thereby eliminating the circulatory and other factors. Using ring preparations of the trachea and intrapulmonary bronchi it has been found that histamine fails to constrict these structures and may under favourable circumstances actually relax them. Since it has been shown above that the air obstruction with histamine is due to a direct

action of the drug on some portion of the respiratory tract, it must be assumed that the more peripheral portions of the respiratory passages, namely the bronchioles, are constricted by the drug. This then is the chief cause of the respiratory obstruction seen in cats after histamine, although other factors may occasionally serve to increase the effect.

Obstruction of the air passages is also one of the chief symptoms seen in anaphylactic shock, and the guinea-pig again is much more affected than the cat. In this and in other respects the symptoms of anaphylactic shock very closely correspond to those produced by histamine, and the author therefore suggests that the air obstruction in both conditions can be explained on the same basis, viz. by an active constriction of the trachea, bronchi and probably bronchioles in the guinea-pig, and by constriction of only the bronchioles in the cat. Experiments on anaphylaxis are being carried out to decide these points.

In man the anaphylactic response is seldom fatal, *i.e.* it is relatively weak when compared with the severe reaction produced in the guinea-pig [see Sollman, 1926, p. 483]. In other words, the anaphylactic response in man resembles that seen in the cat rather than that of the guinea-pig, and it is possible that the air obstruction in this condition in man may prove to be due, as in the cat, to a constriction limited to the bronchioles. It may be found that this hypothesis also serves to explain the air obstruction that occurs in the closely related asthmatic attacks of man.

It is of interest to compare the actions of histamine on the respiratory passages of the cat as described above with the effects of the drug on the blood vessels of this animal [Dale and Richards, 1918]. Thus, while the trachea and bronchi are relaxed by histamine, the bronchioles or finer tubules are constricted; whereas, while the arterioles are constricted by histamine, the capillaries or finer vessels are relaxed. In both cases, however, the peripheral effects of the drug completely overshadow the other actions. It is of interest also to note that the only other mammalian tissue which is relaxed by histamine is the rat's uterus [Guggenheim, 1912].

Summary.

Using various methods of investigation it has been shown that histamine produces obstruction of the respiratory passages of the guinea-pig by a direct constrictor effect on the trachea and bronchi. The bronchioles probably also take part in the action. In the cat histamine causes obstruction chiefly by a direct constrictor effect on the bronchioles. The bronchi and trachea appear to take no part in the obstruction, for they may actually be relaxed by histamine.

A method has been described for recording the effects of drugs on isolated ring preparations of the trachea and bronchi of the commonly employed laboratory animals, viz. the cat, rabbit and guinea-pig.

The author wishes to express his thanks to Prof. J. W. C. Gunn of the Department of Pharmacology, University of Cape Town, for the interest taken in this work, and to Mr J. W. Bates, technical assistant, for any diagrams shown.

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"CHLORIDE SECRETING CELLS" IN THE GILLS OF FISHES, WITH SPECIAL REFERENCE TO THE COMMON EEL.

By ANCEL KEYS[1] and E. N. WILLMER.

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Introduction.

The recent discovery of the chloride secretory activity of the gills of the eel [Keys, 1931*b*] demanded consideration of the histology of the gills. No anatomical basis was known to which the secretory activity might be ascribed and the view was firmly entrenched that respiratory epithelia in general are uniformly devoid of secretory elements. As Winterstein [1921, p. 256] puts it: "Nirgends ergibt sich ein morphologischer Anhaltspunkt für eine 'sekretorische' Tätigkeit, nirgends zeigt das respiratorische Epithel die charakterischen Eigenschaften sezernierender Zellen."

It was shown [Keys, 1931*b*; Bateman and Keys, 1932] that the chloride secretion exhibited by the gills of the eel in sea water is an active process requiring the expenditure of large amounts of energy. The possibilities would seem to be:

(1) A simple respiratory epithelial membrane capable, in the eel, of accomplishing an amount of osmotic work comparable, on the basis of equal weights of tissue, with that done by the mammalian kidney, or

(2) Special secretory cells present in the gills, implying error in the older studies.

The present paper is a report of investigations which revealed the undeniable existence of large numbers of secretory cells in the gills of eels and other teleosts. The constant appearance of great numbers of these cells so situated in the gills as to have access to both internal and external media, combined with the proof that they are not mucous cells, gives them an unusual physiological interest.

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Methods.

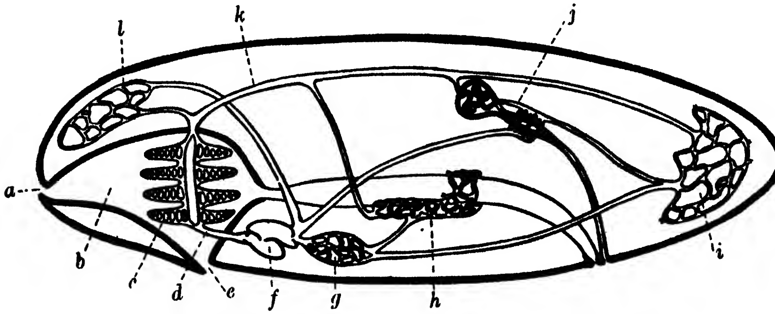
The macroscopic anatomy of the branchial circulatory system in the eel was studied by perfusion methods [Keys, 1931*a*] and by dissection of anæsthetized eels, pithed eels, and fixed and injected specimens. For the investigation of the course of the blood vessels in the gills four methods were used. The main efferent vessels of the gills were tied off so that the gill vessels, which thus became engorged with blood cells, could then be easily identified in sections. Secondly, the vessels were perfused with Indian ink dialysed against Ringer's fluid, and this method gave the clearest picture of the way in which the vessels traverse the filaments. Thirdly, the method of injection of silver nitrate and subsequent reduction was used to impregnate the endothelial lining of the vessels. Finally, the course of blood flow through the gills in the anæsthetized animal was observed microscopically with transmitted light.

The gills for microscopical investigation were fixed in Bouin's or Zenker's fluids or with Flemming's fluid from which the acetic acid was omitted. Attempts were made to fix the tissues by perfusion with 4 p.c. neutral formalin, but the results were unsatisfactory as a considerable amount of shrinkage occurred in the cytoplasm, especially in the cytoplasm of the secretory cells to be described. Sections were stained with hæmatoxylin and eosin or hæmalum and eosin, and these, after fixation in Bouin's fluid, produced the most satisfactory histological pictures. Mallory's triple stain and Giemsa's azure eosin were also used with success. A considerable number of sections were treated with metachromatic thionin to establish the positions of mucous cells. The finer structure of the cells was studied chiefly in cells fixed with Flemming's fluid (without acetic acid) and stained with Heidenhain's iron hæmatoxylin.

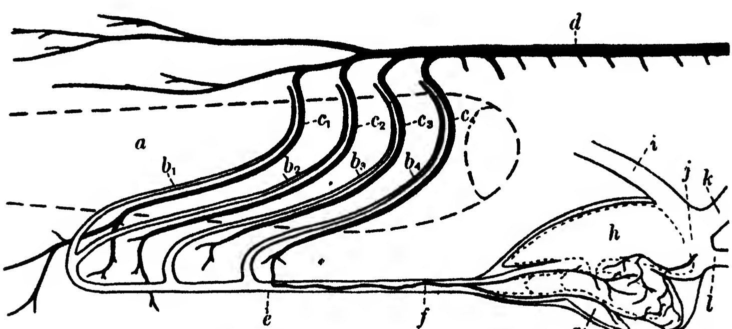
The anatomy of the branchial circulatory system.

The arrangement of the general and the branchial circulatory systems in the eel is typical of the teleost fishes. Text-fig. 1 is a diagrammatic representation of the general circulation in the teleost. Venous blood from the anterior and posterior cardinal systems passes through the ductus cuvieri and joins the blood from the hepatic vein in the sinus venosus. From there the blood traverses, in succession, the auricle, the ventricle, bulbus arteriosus, ventral aorta, the gills and thence to the dorsal aorta. The details of the circulation in the gills will be described later in this paper.

A less diagrammatic picture of the branchial circulation is given in Text-fig. 2 in which oxygenated blood is represented in black. The gills of the right side are not shown. The arterial supply to the tissues of the head is derived from small branches of the anterior efferent vessels of



Text-fig. 1. Essential circulatory system (schematic). *a*, mouth; *b*, branchial chamber; *c*, branchial vessels; *d*, ventral aorta; *e*, branchial aperture; *f*, heart; *g*, hepatic circulation; *h*, capillaries of alimentary canal; *i*, capillaries of posterior part of body; *j*, kidney; *k*, dorsal aorta; *l*, capillaries of anterior part of body.



Text-fig. 2. Branchial circulatory system. *a*, branchial chamber; b_1, b_2, b_3, b_4, afferent branchial vessels; c_1, c_2, c_3, c_4, efferent branchial vessels; *d*, dorsal aorta; *e*, ventral aorta; *f*, coronary artery; *g*, ventricle; *h*, auricle; *i*, anterior cardinal sinus; *j*, sinus venosus; *k*, posterior cardinal sinus; *l*, hepatic vein.

the gills, and the venous drainage from the head tissues is collected by the anterior cardinal system. We did not find a highly developed hypobranchial system such as is present in some elasmobranchs [Keys, 1928].

We have confirmed the description of the coronary system given by Grant and Regnier [1926]. The left coronary artery is shown, in Text-fig. 2, traversing the ventral aorta posteriorly to the heart; the

distribution of the vessels on the heart itself is extremely variable. As Grant and Regnier found, the coronary veins drain into the sinus venosus.

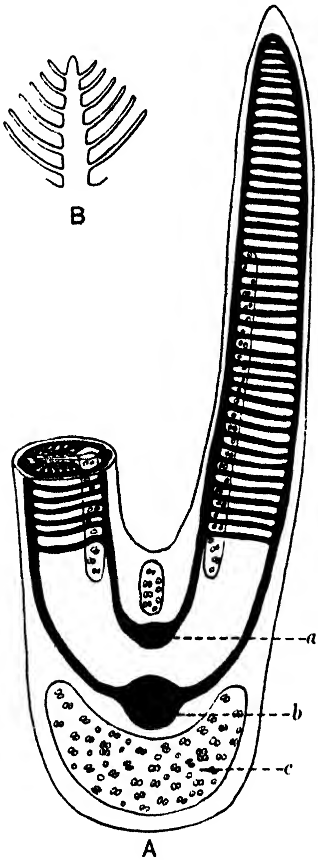


Fig. 3.

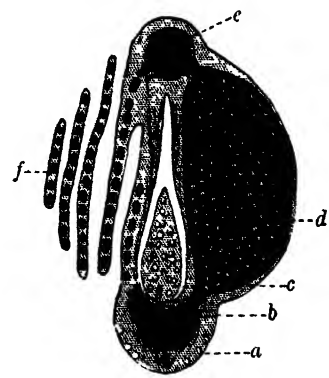


Fig. 4.

Text-fig. 3. A. Diagrammatic section through gill bar showing arrangement of vessels. *a*, afferent vessel; *b*, efferent vessel; *c*, cartilage. B. Diagram showing arrangement of leaflets at the tip of a filament.

Text-fig. 4. Diagrammatic section across a filament. *a*, mucous cells, *b*, afferent blood vessel; *c*, cartilage; *d*, leaflet cut parallel to its surface; *e*, efferent blood vessel; *f*, leaflets cut across.

The eel, like the other teleosts generally, possesses four branchial bars on either side, and each bar bears, on its outer side, a double row of filaments. Each filament, in turn, bears on each side a row of lamellæ or respiratory leaflets which provide the surface for both respiratory

and osmotic exchange. Text-fig. 3 shows a transverse section through a gill bar and also a diagram of the tip of a filament viewed at right angles to the gill bars.

As described by earlier workers [see Oppel, 1905], each filament on the bar is supported for about two-thirds of its length by a small rod of cartilage (the gill ray) which is situated on the inner side. The afferent branchial artery (which carries venous blood) courses along the external side of the gill bar in the V between the filaments (see Text-fig. 3) and sends a branch to each filament which runs along the gill ray on the inner side. At the level of each leaflet this filament arteriole gives off a small branch which opens into the leaflet. The leaflet consists of an upper and lower respiratory epithelium, each composed of a single layer of flattened cells; between the upper and lower respiratory epithelia lies the system of blood channels. The blood is enclosed between two membranes kept apart by somewhat columnar cells (the pilaster cells of Bietrix [1895 *b*], see Oppel, p. 23 *et seq.*) as a roof might be supported by a colonnade of pillars. These supporting cells are expanded at each end and it is these expansions which, by fusing together, give rise to the two membranes. The nuclei of the supporting cells are situated almost invariably in the middle region of the cell. Between the respiratory epithelium and the blood channels there is some evidence, at least in places and especially towards the base of the leaflets, for the existence of a true basement membrane such as has been described by previous workers [see Oppel, 1905].

After the blood has flowed through the leaflet it is collected again on the outer side of the filament and flows into an efferent vessel which traverses the outer margin of the filament to the gill bar, receiving the blood from each leaflet which it passes in its course. The arrangement of the blood channels is illustrated in Text-fig. 4, in which the black represents blood and the lighter areas show the tissue substance. The leaflet on the right in this figure has been cut in a direction parallel to its flat surface so that the supporting cells are clearly shown; on the left are seen leaflets cut at right angles to their flat surfaces.

The more minute histological structure is shown in Text-fig. 5 and on Plates I and II. In the outer regions of the leaflets the typical respiratory arrangement is seen in which a simple respiratory membrane covers the blood vessels. The separate character of this membrane, as distinguished from the membrane which forms the blood channels, is indicated. At the base of the leaflets, however, the picture changes and the epithelium thickens into a type conforming more to stratified

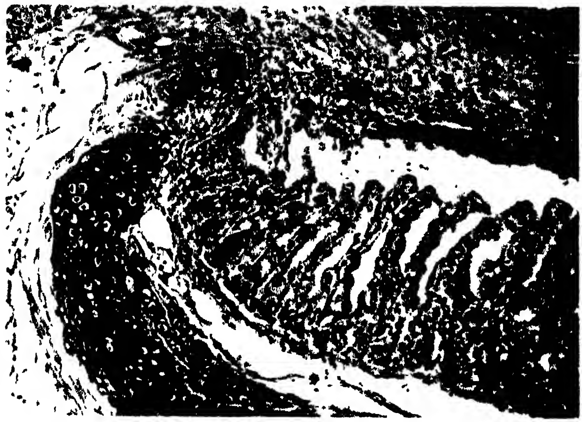


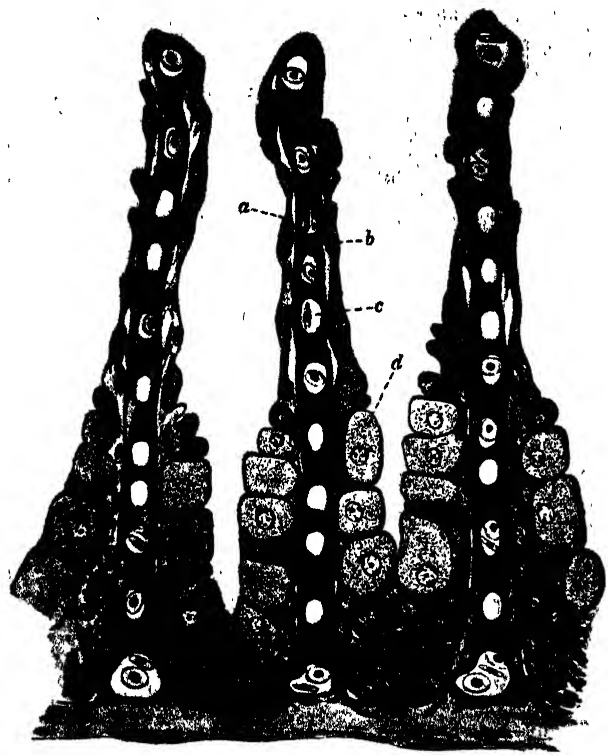
Fig. 1. Microphotograph (× 170) showing the position of the "chloride secreting" cells at the base of the leaflets (hæmalum and eosin).

Fig. 2. Microphotograph (× 400) of leaflets with "chloride secreting" cells (hæmalum and eosin).

Microphotograph (× 880) of "chloride secreting" cells
(iron hæmatoxylin, Heidenhain).

epithelium which in turn lies on a definite basement membrane of connective tissue. The latter is penetrated periodically by the blood vessels entering or leaving the leaflet.

In the proximal portions of the leaflets, particularly on the side nearest to the gill rays and therefore near to the blood vessels which



Text-fig. 5. Diagram showing the position of the "chloride secretory" cells. *a*, respiratory epithelium; *b*, "pilaster" cell; *c*, blood channel; *d*, "chloride secretory" cell.

bring blood to the leaflets, there are found large numbers of cells of a very different character from those forming the ordinary epithelium. The nucleus, instead of being more or less ovoid is almost spherical and is much larger; typically, it contains one nucleolus and a thin network of chromatin. Often it occupies a more or less central position in the cell, but sometimes the whole cell is slightly elongated and approaches the columnar form in which case the nucleus is situated towards the base of the cell.

The cytoplasm of these cells is finely granular and has a greater affinity for eosin than the rest of the epithelium. The cells are therefore conspicuous in sections stained with hæmalum and eosin or with Giemsa's azure eosin. The cells are typically ovoid in shape and may occupy the whole epithelial thickness, but they never, or only very rarely, appear to be more than one layer thick. They are more particularly confined to the region of the base of the leaflets, but isolated cells of the same character have been observed occasionally on the outer regions of the leaflets. In a single cross-section of the leaflets on the gills of the eel up to eight or ten of these cells may be found between a single pair of leaflets if the section has been cut in the neighbourhood of the gill ray. In other regions they are generally less numerous. The diameter of these cells is on the average 15μ, and the diameter of the nucleus about 6μ; both cell and nucleus are very markedly larger than the epithelial cells and their nuclei.

The presence of these cells of a definite secretory type on the leaflets of the gills and the experimentally found secretion of chloride by the gills at once suggested that the two may be intimately connected. Their position on the leaflets near the gill rays is interesting and may be significant. If these cells are doing a considerable amount of work it is probable that their demand for oxygen is high, so that they would tend to lower the oxygen saturation of the blood or fluid with which they are in contact. If they were situated elsewhere on the leaflets the blood would tend to leave the gills not fully saturated and by concentrating the cells around the afferent vessels this contingency is avoided; the blood can become saturated with oxygen after passing the secreting cells, and these can draw oxygen from the outside fluid as well as, or instead of, from the blood.

A number of other species were studied in order to see if there is any correlation between the presence or absence of these cells and the probable chloride-secreting capacity of the gills as inferred from the habitats of the various species. In the fishes so far studied none has shown as many of these specialized cells as occur in the eel, but most of them have exhibited cells of the same type although their form may vary somewhat. The findings with the various species have been condensed in Table I. The distinguishing criterion applied here to these cells was eosinophilia in very large cells with nearly spherical nuclei. As shown in the table, the character of the cells is somewhat variable, and it may be that there are forms intermediate between the true epithelial cells and those of the secretory type. This fact sometimes makes it difficult

to decide whether or not specialized secretory cells do occur, particularly in the fresh-water species where the presence of mucous cells between the leaflets adds a source of difficulty.

TABLE I.

Species	Number of cells	Description of cells and other notes
Anguilla vulgaris (the common eel)	Abundant between the leaflets, especially at their bases	Large round or ovoid cells, nearly spherical nucleus situated near the centre of the cell
Conger vulgaris (conger eel)	Two or three per section between each leaflet	Rather more columnar in shape than in the common eel, and the nucleus tends to be towards the base of the cell
Salmo salar (salmon)	Fairly numerous between the leaflets in adult	Smaller than in the common eel but well developed
Pleuronectes platessa (plaice)	Few except near the gill ray where they are well developed	Nuclei small and more condensed than in the eel
Cottus bubalis	A few	Rather small
Crenilabrus melops	Cells abundant	Well developed but nucleus rather small. Ordinary epithelium somewhat columnar
Scyliorhinus canicula (the rough dogfish)	None observed	Mucous cells abundant, also occurring between the leaflets
*Leuciscus rutilus** (roach)	Very few if any	Eosinophilic cells occur under the epithelium at the sides of the filaments
*Gobio fluviatilis** (gudgeon)	Cells fairly abundant, especially at the base of leaflets, in some sections, absent in others	Mucous cells occur among the eosinophilic cells
*Leuciscus vulgaris** (dace)	Very few	—
*Abramis blicca** (bream)	Fairly well developed at the base of the leaflets	Generally more tissue and mucous cells between the leaflets. The secretory cells do not have contact with both blood and external medium

* These are fresh-water teleosts.

In the eel and in several other species examined, the cells do not show any definite polarity, the nucleus being somewhat centrally placed in an ovoid cell. This may possibly be taken as an indication that the cells may be capable of working in either direction according to the osmotic concentrations. Incidentally there is a very striking histological resemblance between these cells and the oxyntic cells of the mammalian stomach whose function is the secretion of chloride, but in this case the cation is hydrogen ion, whereas in the eel the cation is sodium or potassium.

Mucous cells of the goblet cell type were also found on the gill filaments in all the species examined. In the marine teleosts and the eel these mucous cells were limited to the filament stalk itself, but in the fresh-water fishes and in the dogfish mucous cells occurred in abundance between and at the base of the respiratory leaflets.

Discussion.

It is rather surprising that the secretory cells described here were not reported earlier. It is true that they are generally limited to certain regions in the gill leaflets and may be entirely absent in some species. However, they are so clearly evident in properly fixed specimens that it seems probable that bad fixation may have been one reason why they were not noticed before.

These cells are present in large numbers in the eel; in animals of about 250 g. weight we estimated between three and six million cells in the gills of each eel. It is obvious that we have here a possible and even probable histological basis for the branchial chloride secretion. These cells, which we shall tentatively term chloride-secreting cells, are not only of an unmistakable secretory type and are not mucous cells, but they are located so as to be in close contact both with the blood and with the external medium. They are undoubtedly more pronounced in the eel than in the other species examined. The eel has an extraordinary power of osmotic regulation, and this may be ascribed largely to its proven ability to secrete chloride from the gills.

Evidence has been presented [Keys, 1931*b*; Smith, 1932; Keys, 1932] for the probability that the marine teleosts generally secrete chloride from the gills. The inference follows that the "chloride-secreting cells," if they really possess this function, should be present in all the marine teleosts; as has been shown, this expectation is realized and we found these cells in all the marine teleosts examined. In an elasmobranch, the dogfish, which is not dependent on a branchial secretory mechanism for its osmotic regulation [see Smith, 1931], we were unable to find chloride-secreting cells.

The salmon possesses "chloride-secreting cells" in a fairly well-developed condition, at least this was the case in the gills of a specimen which was captured while passing from sea water to fresh water prior to spawning. In a very young specimen taken from fresh water no trace of the cells could be found. This specimen, however, was fixed with formalin which has since been shown to be unsuitable for the

demonstration of this type of cell since it causes very pronounced shrinkage of the cytoplasm.

The presence of these cells in some of the fresh-water teleosts admittedly complicates the histological interpretation. However, it will be noted (Table I) that these cells are generally less abundant in the fresh-water teleosts than in the marine forms. They may, of course, be non-functional in the fresh-water species. Another possibility is that these cells are so organized that they do not necessarily secrete chloride out of the blood but that they always secrete in opposition to the concentration gradient. If this were the case they would tend, in the fresh-water fishes, to help maintain the salt content of the blood which is continually in danger of depletion in the fresh-water fishes.

A thorough survey of the histology of the gills of fishes from various habitats will be required before final conclusions can be drawn, but we feel justified in suggesting that the "chloride-secreting cells" described here are associated functionally with the branchial chloride secretion in the eel, at least, and probably in other marine teleosts.

From an evolutionary point of view it is perhaps interesting to note that the occurrence of mucous cells between the leaflets seems to be confined to the dogfish (the only elasmobranch examined) and the fresh-water species. They appear to have been lost in the essentially sea-water forms.

SUMMARY.

The anatomy of the branchial circulation in the eel (*Anguilla vulgaris*) is described.

The histology of the gills of ten teleost species (including the eel) and of the dogfish (*Scyliorhinus canicula*) was studied. Cells of a secretory type were found to be present in large numbers in the gills of the eel and in smaller numbers in the gills of other teleosts; they were not found in the dogfish.

The possible correlation of these secretory cells with the chloride-secreting activity of the gills is discussed.

We are indebted to Mr J. Gray, Dr J. B. Bateman, Mr F. C. Stott and Mr F. P. Coyne for many of the specimens used in this work.

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THE DIURETIC ACTION OF ALCOHOL AND ITS RELATION TO PITUITRIN.

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THAT alcohol has a diuretic action is well known, but the way in which it produces its effect is not fully understood. Alcohol put into the circulation of a heart-lung-kidney preparation causes a cessation of urine flow according to Loewy and Bornstein [1927]. Hence it would seem probable that its action as a diuretic is extrarenal.

The experiments to be described represent an attempt to investigate the mechanism of alcohol diuresis. They fall into two series:

(I) Experiments on normal human subjects.

(II) Experiments on cats under amytal.

PART I. EXPERIMENTS ON HUMAN SUBJECTS.

Firstly, the diuretic effects of alcohol and certain of the more common diuretic drugs were compared. All experiments were carried out under comparable conditions; that is, at constant room temperature first thing in the morning, the subjects having no breakfast. In every experiment the volume of liquid taken was 300 c.c. at 29° C. Control experiments were done, water alone being drunk. The results in Table I show the comparative diuretic effect of alcohol (10 p.c. by volume), urea (5 p.c.), diuretin (theobromine sodium salicylate, 0·3 p.c.) and whey (made by clotting milk with rennin). It can be seen from these figures that alcohol has a very definite and rapid diuretic action. Adolph and Ericson [1926] considered that there are three types of diuresis: (1) water diuresis due to hydræmia, (2) saline diuresis, (3) drug diuresis. The rapidity of action of alcohol is like simple water diuresis. This latter is due to hydræmia and the resulting lowered osmotic pressure. It is antagonized by the antidiuretic hormone of the pituitary.

Experiments were carried out to show the relation of pituitrin to alcohol diuresis. Four different types of experiments were performed,

TABLE I.

No. and type of exp.	Urine flow per ½ hour before, c.c.	Urine flow in successive ½ hours after, c.c.				Total in 2 hours after, c.c.
Subject A						
31. Water, 300 c.c.	15	28	155	115	18	316
27. do.	19	50	216	136	12	414
24. do.	11	21	140	95	11	267
25. Alcohol, 300 c.c. 10 p.c.	17	19	134	214	148	515
28. do.	25	91	227	256	120	694
18. Urea, 300 c.c. 5 p.c.	15	Collected at the end of 2 hours				165
Glucose, 300 c.c. 25 p.c. (av. of 4 exps.)	16	do.				230
Glucose, 300 c.c. 25 p.c. and 10 p.c. alc. (av. of 6 exps.)	18	do.				444
Subject B						
24. Water, 300 c.c.	26	112	202	29	24	367
23. do.	18	56	233	52	10	351
25. Alcohol, 300 c.c. 10 p.c.	22	22	126	230	124	502
27. do.	16	68	235	265	43	611
10. Diuretin, 1 g. in 300 c.c. water	25	Collected at the end of 2 hours				365
Glucose 300 c.c. 10 p.c. (av. of 4 exps.)	21	do.				260
Glucose, 300 c.c. 25 p.c. and 10 p.c. alc. (av. of 6 exps.)	22	do.				485

all under the conditions mentioned above. The urine was collected every half-hour till the rate was constant, then an experiment of one of the following types was performed:

(A) 300 c.c. of tap water at 29° C. was drunk.

(B) 300 c.c. of tap water at 29° C. was drunk and an injection of 0·1 unit pituitrin was given.

(C) 300 c.c. of 10 p.c. alcohol at 29° C. was drunk.

(D) 300 c.c. of 10 p.c. alcohol was drunk and an injection of 0·1 unit pituitrin was given.

Subsequently the urine was collected at half-hourly intervals till the rate had returned to normal again. The results of these experiments are set out in Table II.

The pituitrin was Parke-Davis's posterior lobe extract (10 units per c.c.) diluted with sterile water 1 in 20 so that an injection of 0·2 c.c., which was the usual dose given, was equal to 0·1 unit. The small dose given in two experiments was half this (0·05 unit). The pituitrin was injected subcutaneously.

The results of these experiments show again the marked diuretic action of alcohol and also that this diuretic effect is able to overcome to a certain extent the antidiuretic effect of pituitrin. This point is brought

out by a comparison of Exps. 35 and 34, the small dose of pituitrin has a considerable effect on water diuresis. In Exp. 35 the time of maximum excretion is delayed by 1½ hours, the time of return to the normal rate by 1½ hours and the total excretion decreased by 110 c.c. In Exp. 34 the effect of the small dose is certainly weaker, the time of maximum excretion and the return to normal are only delayed by ½ hour and the total excretion only decreased by 27 c.c.: that is, the pituitrin is almost ineffective. It seems therefore that the alcohol antagonizes the antidiuretic action of pituitrin.

TABLE II.

No. and type of exp.	Urine vol. in c.c. per ½ hour: Before	Urine vol. in c.c. per ½ hour: Successive ½ hours after	Time of max. excretion (hours)	Time to return to normal (hours)	Total excretion till return to normal (c.c.)
Subject A					
31. A. Water diuresis	15	28, 155, 115, 18	1	2	316
27. do.	19	50, 216, 136, 12	1	2	414
30. B. Water, pituitary injection	16	14, 15, 13, 10, 19, 20, 19, 30, 61, 8	4½	5	209
25. C. Alcohol diuresis	17	19, 134, 214, 148, 6	1½	2	521
26. do.	25	91, 227, 256, 120, 21	1½	2	715
29. D. Alcohol, pituitary injection	13	13, 10, 11, 14, 75, 160, 58, 31, 21	3	4½	393
32. do.	11	12, 12, 11, 9, 85, 147, 128, 60, 28	3	4½	492
Subject B					
24. A. Water diuresis	26	112, 202, 29	1	1½	343
23. do.	18	56, 233, 52, 10	1	2	351
28. B. Water, pituitary injection	16	15, 18, 17, 20, 14, 17, 36, 38, 9	4	4½	184
29. do.	20	17, 25, 16, 10, 21, 39, 59, 25, 6	3½	4½	218
35*. do.	30	25, 16, 16, 14, 135, 22, 10	2½	3½	238
25. C. Alcohol diuresis	22	23, 126, 230, 124, 15	1½	2½	518
27. do.	16	68, 235, 265, 43, 17	1½	2½	628
33. D. Alcohol, pituitary injection	23	23, 15, 17, 22, 121, 42, 58, 15	3	4	313
34*. do.	20	30, 30, 126, 273, 69, 18	2	3	546

* Indicates where small dose of pituitrin was used, Exps. 34 and 35.

This could be brought about in several ways. We know from the work of Krogh that one of the most important functions of the posterior lobe of the pituitary gland is to maintain a normal degree of permeability of the capillaries. The absence of pituitrin in a perfusion liquid causes their permeability to increase. An injection of pituitrin into a normal animal probably makes the capillaries more impermeable. This may be the explanation of the antidiuretic effect of pituitrin in suppressing water diuresis. The blood is diluted to the same extent as in water diuresis [Priestley, 1921], but the urine is scanty because the water cannot be filtered through the glomerulus. Now the diuretic action of alcohol and

its antagonism to the antidiuretic action of pituitrin could be explained in several ways:

(1) That alcohol causes a greater hydræmia than water and thereby can overcome the impermeability due to pituitrin.

(2) That alcohol actually destroys or inhibits the secretion of the capillary substance of the pituitrin.

(3) That alcohol increases capillary permeability directly.

(4) That alcohol increases kidney volume and blood flow.

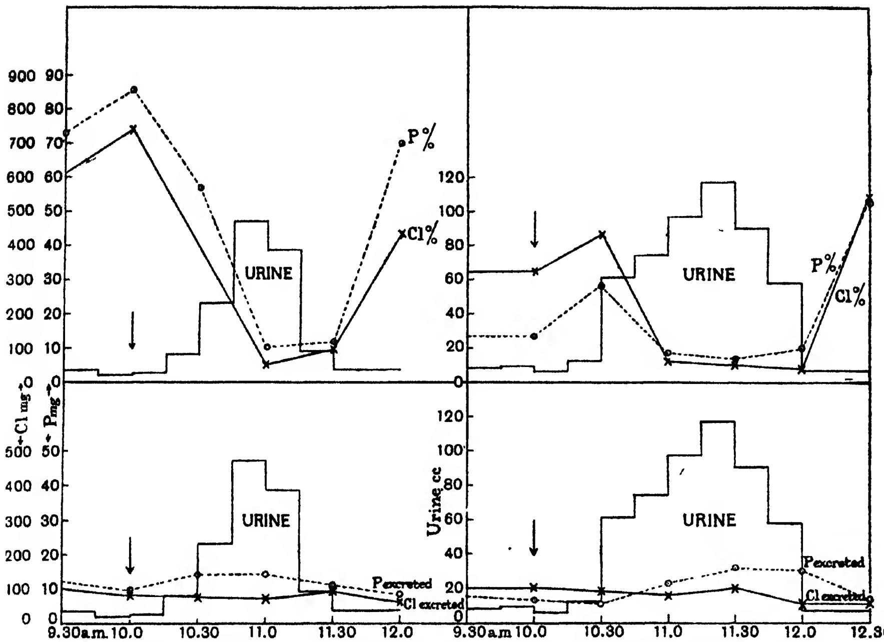
(5) That alcohol increases diffusion pressure in the glomerulus by increasing the blood-pressure, so that the decrease in permeability due to pituitrin is overcome.

Taking the last consideration first, the rise in blood-pressure from such a relatively small dose of alcohol is very slight or non-existent and, if this were the reason for alcohol diuresis, then a similar effect should have been obtained when alcohol was injected into anæsthetized cats in doses sufficient to give blood-pressure rise of about 10 mm. Hg. Here the alcohol did not produce diuresis. The experiments to show this are quoted in more detail later. Alcohol did not cause diuresis in the experimental animals, so the fourth point could not be tested.

Since the result of all the other three possibilities is ultimately the same, these effects cannot be dissociated. It is generally accepted that alcohol increases cell permeability. There is so far no good evidence that alcohol inhibits the secretion of the pituitary gland, but that alcohol and pituitary functions seem to be antagonistic was suggested by Edkins and Murray [1931]. With regard to the possible hydræmic effect of alcohol, attempts were made to investigate this by determining the osmotic pressure of the blood in these experiments, but the results were not consistent. Analyses of the urine were made in all cases, the results of four typical experiments are given in Figs. 1 and 2.

The variations in concentration seem to depend entirely on the urine volume, that is, alcohol causes an exaggerated water diuresis. The output of chloride and phosphate did not suffer much variation, though there was generally a small increase in phosphate output. In subject B there was a slight rise in chloride output and a more marked increase of phosphate; this was probably due to the very rapid rate of secretion.

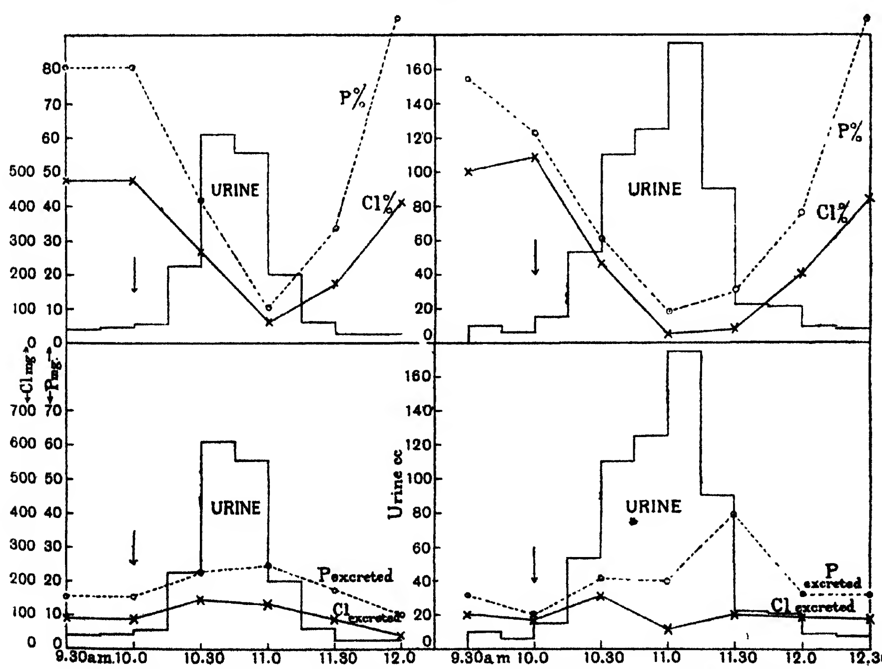
The graphs of the urine excretion for alcohol experiments and simple water ingestions are very similar, suggesting a similar type of diuresis.



Subject A. Water diuresis, Exp. 24

Subject A. Alcohol diuresis, Exp. 25

Fig. 1.



Subject B. Water diuresis, Exp. 23

Subject B. Alcohol diuresis, Exp. 27

Fig. 2.

PART II. EXPERIMENTS ON CATS UNDER AMYTAL.

Because of the reverse effects of pituitrin on the urine secretion in anæsthetized animals, as compared with unanæsthetized animals, a series of experiments was done to investigate the effect of alcohol on the urine secretion in cats under amytal, and the relation of alcohol effects and pituitrin injection in these animals. In all experiments the bladder was catheterized and the original rate of urine secretion determined. First of all control experiments were made, giving warm water by means of a tube tied into the œsophagus, to determine whether a water diuresis could be established in these animals. In most cases 20 c.c. water were given, this is for the average sized cat (2·7 kg.), 8 c.c. water per kg. body weight which is much more proportionally than 300 c.c. for a human subject averaging 60 kg., making 5 c.c. per kg. body weight. The figures given in Table III show that there is no diuresis after water ingestion in cats under amytal. In other experiments, alcohol (20 c.c. of 20 p.c.) was given, again a bigger dose than was given to the human subjects. Here again there is no diuresis as seen by the figures in Table III.

TABLE III. The secretion of urine in cats after administration of:

A. Water 20 c.c.

No. of exp.	Urine before per ½ hour c.c.	Urine 1st ½ hour after c.c.	Urine 2nd ½ hour after c.c.
20	1·2	1·3	1·1
23	0·8	1·0	0·45
24	1·6	1·4	1·0
26	0·6	1·0	0·8
27	0·7	0·8	0·7
30	2·0	3·6	

B. Alcohol 20 c.c. 20 p.c.

No. of exp.	Urine before per ½ hour c.c.	Urine 1st ½ hour after c.c.	Urine 2nd ½ hour after c.c.
20	1·2	1·2	0·4
23	1·0	1·3	1·4
24	1·9	1·9	2·0
27	1·75	0·7	0·7
12	4·0	3·5	4·8
13	3·8	3·6	
16	2·7	2·0	2·0
19	0·5	0·4	0·5
22	1·6	1·0	0·9
28	0·3	0·2	0·4
31	2·7	2·3	2·0
37	1·0	0·9	0·9
38	0·3	0·3	0·3

In two of these alcohol experiments a record of the blood-pressure was taken. A slight rise in blood-pressure was noted after giving the alcohol. It seems that the kidney in anæsthetized animals is not capable of responding to a slight degree of hydræmia produced by water ingestion.

These animals responded in the usual way to pituitrin injections. Giving a dose of 0·5–0·75 c.c. of pituitrin intravenously to the animals

1 hour after the administration of water the following results were obtained (see Table IV, column A). Fifteen such experiments were performed, giving an average increase of urine in the first hour after injection of 13·4 c.c. This increase is to be compared with the figures in Table IV,

TABLE IV. Effect of pituitrin on diuresis in cats under amytal.
Increase in urine excretion 1 hour after injection of 0·5 to 0·75 c.c. P.D. pituitrin.

	A. After 20 c.c. water ingestion		B. After ingestion of 20 c.c. 20 p.c. alcohol	
No. of exp.	A (i) 1st injection pituitrin c.c.	A (ii) 2nd injection pituitrin c.c.	B (i) 1st injection pituitrin c.c.	B (ii) 2nd injection pituitrin c.c.
22			0·0	
12	15·0	—	2·6	
13	19·0	—	−2·3 (decrease)	
16	7·0	—	6·2	
20	13·5	—	1·0	
23	15·5	—	8·7	
24	23·0	—	14·2	
27	13·0	—	2·3	
Average (7)	15·0	—	4·1	
38	—	—	—	3·3
37	—	—	—	7·2
Average	—	—	—	5·0
8	4·7	5·1		
14	13·0	3·5		
15	11·0	12·0		
20	13·5	16·0		
32	9·0	4·8		
33	19·2	6·0		
35	8·9	12·2		
36	15·4	15·0		
Average (8)	11·8	9·4		
Average of all A (i) exps. (15)	13·4			

column B, where the injection of pituitrin after the giving of alcohol gave a urine increase in the first hour on an average (eight experiments) of 4·1 c.c. Since these results after alcohol were obtained on a second injection of puitrin into animals which had previously given a diuresis for a first dose, the point might be raised that there was less diuresis after alcohol because it was a second dose of pituitrin. To show that this was not the cause of the decrease in effect of the pituitrin, experiments showing the result of two successive injections after water ingestion were performed. The results of these are also given in Table IV, and an average of eight experiments gave 11·8 c.c. increase for the first and 9·3 c.c. urine increase for the second dose. In these cases no further water was given

before the second injection of pituitrin. Hence it is seen that the decreased effect of pituitrin after alcohol is not due to the fact that it is a second injection. It would appear therefore that whatever is the mechanism of pituitrin diuresis in these animals, it is antagonized by previous ingestion of alcohol. The concentration of alcohol in the blood in the animals was estimated in many cases, the usual concentration was 25 mg. per 100 c.c. blood.

Now whether these two sets of results:

(*a*) those on human subjects showing that alcohol is a potent diuretic and has an action antagonistic to the antidiuretic substance of pituitrin,

(*b*) those on cats in amytal anæsthesia showing that alcohol has no diuretic effect in these animals and that it antagonizes the diuretic action of pituitrin,

can be correlated is very difficult to say.

If alcohol antagonizes both antidiuretic and diuretic action, and, as will be shown in another communication, it also inhibits pituitrin hyperglycæmia, its effect seems to be very general. It also appears that alcohol, besides being able to antagonize these hormonic effects on blood sugar when pituitrin is injected, can also possibly antagonize the normal effect of the pituitry gland in these respects as well [Edkins and Murray].

Summary.

1. The diuretic action of alcohol on normal human subjects was compared with that of certain recognized diuretic substances and found to have a rapid and powerful diuretic action.

2. The diuretic effect of alcohol antagonized that of an injection of pituitrin in normal human subjects.

3. It is considered from the results that the effect of alcohol is an exaggerated water diuresis, not a specific effect on the kidneys.

4. In amytalized cats alcohol does not act as a diuretic.

5. Alcohol antagonized the diuretic effect of pituitrin injection in these cats.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

III. The relationship of blood phosphorus to liver glycogen and blood glucose in the decapitate cat.

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In their investigation of the carbohydrate metabolism of the fasting cat after decapitation, Evans, Tsai and Young [1931] concluded that the restitution of liver glycogen was greater than could be accounted for as occurring wholly from blood sugar or lactate, or both of these substances. Nevertheless it appeared to be significant that in its restoration the amount of liver glycogen tended to approach, but rarely if ever to exceed, that initially present in the liver at the beginning of the experiment, though that amount was often very different in different individuals. It was attractive to suppose that during decapitation the liver glycogen was converted into some intermediate substance which perhaps found its way temporarily into the circulation, to be ultimately returned in whole or in part to the liver and there reconverted into glycogen. This possibility was passed over by Evans, Tsai and Young in favour of the hypothesis of glyconeogenesis, but largely because there was at that time little to suggest the probable nature of such an intermediary.

Other investigators had felt similar difficulties with regard to the equating of glycogen lost by the liver or muscles [Corkill and Marks, 1930], though Simpson and Macleod [1928] found the discrepancy smaller for liver than for muscle.

The known relationship of phosphoric acid to carbohydrate metabolism, and the important work of Cori and Cori [1931] indicating that under various conditions muscle glycogen may be converted into hexose phosphoric acid, however, made it worth while studying the phosphorus changes of the blood in our decapitate animals.

Further, a formation of hexose monophosphate in glycogenolysis in the liver was indicated by Barrenscheen *et al.* [1930]. Apart from the

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possibility that the liver glycogen might be in part converted into hexose phosphate and retained as such in the liver pending its restoration to glycogen, there was the further possibility that hexose phosphate as well as glucose might find its way into the blood stream: in either case some change in the blood phosphates might be anticipated, and in the latter case it was just conceivable that the hexose phosphate of the blood might be considerable in amount, and since its reducing power is only about two-thirds that of glucose, the apparent inadequate loss of carbohydrate from the blood during glycogen formation might be due to the fact that part of what was estimated as glucose might be a quantity of hexose phosphate corresponding to an amount of glucose 50 p.c. greater.

It was found by Kay [1928] that the plasma never contains appreciable amounts of ester phosphorus. The red corpuscles, however, appear to contain hexose phosphate and glycerophosphate, and these in determinations by the Briggs' method would be estimated as organic phosphorus. According to Kay and Robison [1924] the corpuscles act as reservoirs for these esters which could diffuse from them as required into the plasma. We therefore used whole blood for the analyses, and estimated the inorganic and organic phosphate as a preliminary to a proper fractionation of the latter should the aggregate changes in its amount justify further enquiry. This in fact proved not to be the case.

Methods.

The methods used were in general those described by Evans, Tsai and Young, with the exception that the modified Shaffer-Hartmann method was employed for estimation of the glucose obtained from glycogen. An outline of this valuable method has been given by Murphy and Young [1932], by permission of Prof. Shaffer.

We determined the total phosphorus, inorganic phosphorus and glucose on the blood of cats before decapitation and at frequent intervals after decapitation. Liver samples were also taken for glycogen. The animals were all fasted 44–48 hours before the experiment.

The following modified Briggs' method was used for the phosphorus determinations in order to permit the use of reasonably small blood samples: 2 c.c. of blood were transferred immediately into 8 c.c. of a 1 p.c. solution of trichloracetic acid in a centrifuge tube. After shaking, 1 c.c. of 40 p.c. trichloracetic acid was quickly added and the total volume brought to 12 c.c. with distilled water. After thoroughly shaking, the tube was centrifuged for 10 min. and the supernatant fluid filtered. 5 c.c. of the filtrate were then used for the inorganic phosphorus and two 1 c.c. portions for duplicate total phosphorus determinations.

For inorganic phosphorus, to 5 c.c. of filtrate were added in succession 1·2 c.c. of 6N H_2SO_4: 2 c.c. of 5 p.c. ammonium molybdate, and 1 c.c. of hydroquinone and sodium sulphite solution: the volume was then made up to 10 c.c. and the solutions thoroughly mixed.

For total phosphorus, 1 c.c. of the filtrate was placed in a pyrex boiling tube; 1·2 c.c. of 6N H_2SO_4 added, and a glass ball placed on the mouth. The tube was then boiled on an

electric heater, as described by Stanford and Wheatley [1925] until the contents charred and dense fumes appeared. The tube was then cooled, 0·1 c.c. of Merck's perhydrol added, and the contents boiled again until the solution was clear and all the persulphate had been decomposed. After cooling, to the contents of the tube were added, with thorough mixing, 2 c.c. of ammonium molybdate and 1 c.c. of hydroquinone and sodium sulphite and distilled water to make the volume to 10 c.c.

For the standard 3 c.c. of KH_2PO_4 (1 c.c. = 0·02 mg. P) were made up in the same way as for the inorganic phosphorus determination. After half an hour the readings were made on a Klett microcolorimeter.

RESULTS.

The blood phosphorus in the unanæsthetized cat.

We found as Kay did, that there is a wide range of normal blood phosphorus levels in the cat. In order to get some results for the unanæsthetized animal we performed cardiac punctures on a series of cats; samples can thus be obtained without causing pain or struggling. In four cats our figures were:

Inorganic phosphorus mg./100 c.c.	Organic phosphorus mg./100 c.c.
9·7	10·6
9·3	10·3
7·5	10·6
5·0	9·2
Average 7·9	Average 10·2

With animals under ether anæsthesia the range from animal to animal was very much greater, as the following examples will show:

Inorganic phosphorus mg./100 c.c.	Organic phosphorus mg./100 c.c.
4·8	9·6
8·3	11·8
5·3	6·1
4·1	18·1
8·9	7·6
7·1	11·8

The average for all our experiments for the initial blood samples under ether anæsthesia was 6·5 mg. for the inorganic phosphorus and 11·1 mg. for the organic phosphorus. Kay [1928] found a mean of 5·6 mg. for inorganic phosphorus in cats (presumably anæsthetized). His figures for the ester phosphorus of the corpuscles, 18–31 mg. (mean = 22), would also agree approximately with our figure for whole blood. In general there was a slightly lower value for inorganic phosphorus in the recently anæsthetized than in the unanæsthetized cat. This is in apparent disagreement with Martland and Robison [1924], who found that in rabbits ether anæsthesia and shock caused an increase in the inorganic

phosphate and also in the total phosphate, but after prolonged anæsthesia we obtained results similar to theirs. The greater range of variability in the anæsthetized animals might be due to differences in the depth of anæsthesia at the time when the samples were taken.

In the individual experiments, however, the blood phosphorus level changed very little as will be seen.

The effect of decapitation on the blood phosphorus. Although, on decapitation, we obtained the usual fall in liver glycogen with the concomitant rise of blood glucose, and after decapitation when the glycogen

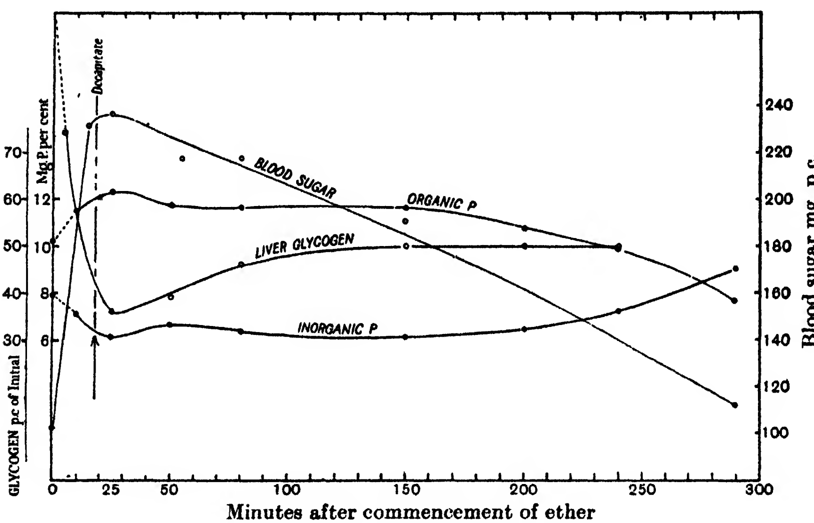

Fig. 1. Decapitate cat. Liver glycogen, blood sugar and inorganic and organic phosphate of blood. Mean curves from ten experiments.

again rose the glucose level fell, the blood phosphorus throughout showed little change. Such alteration as occurred, however, was fairly regular, and is best illustrated by Fig. 1, which represents the mean results of ten typical experiments.

The liver glycogen is expressed as percentage of the calculated original amount present at the commencement of etherization. The blood glucose and organic and inorganic phosphorus are shown as mg./100 c.c. of the whole blood.

It is seen that the inorganic phosphate, which is presumed to have been reduced by ether from about 8 mg./100 c.c. to about 7·1 at the end of 10 min., is further reduced after decapitation to about 6·1. The organic phosphorus shows a change in the opposite direction, from

11·5 mg. after 10 min. under ether, to about 12·3 after decapitation. The glycogen has fallen meanwhile from about 75 p.c. of the initial value to 36 p.c. The blood sugar changes are out of all proportion to the phosphate changes, the total rise of blood sugar from commencement of etherization to about 6 min. after decapitation being about 134 mg./100 c.c. There was a subsequent slight increase in the inorganic phosphate up to about 50 min., and afterwards a slow fall again. The organic phosphorus showed changes in the opposite direction of similar magnitude. During this time there was glycogen recovery.

We have little doubt that the fall of inorganic phosphate is to be explained on the lines indicated by the work of Cori and Cori, viz. a formation in muscle (and possibly in other tissues) of hexose phosphate from glycogen. Whether the rise in the organic phosphate of the blood is indirectly connected with this it is impossible to say. We frequently found, as shown in Fig. 1, that at the end of the experiment when the blood-pressure had fallen the inorganic phosphorus rose to above the initial value and the organic phosphorus fell below the initial level. These changes, however, need not be further considered here, because, owing to the large removals of blood for sampling, there was less complete recovery of glycogen in the later stages than usually occurs; in fact in some of the experiments averaged out in Fig. 1 a considerable fall of liver glycogen occurred in the later periods. Whatever may be the cause of these terminal changes, they probably have no connection with our present problem. It is evident, on the whole, that although considerable changes occur in both liver glycogen and blood sugar after decapitation, the blood phosphorus is not affected to an extent sufficient to justify its being considered as connected with the presence of notable stores of carbohydrate-phosphoric esters in the blood itself.

Effect of anæsthesia and repeated bleeding on the blood phosphorus level. In two experiments cats were kept under ether anæsthesia throughout, and only the usual sampling of liver and blood was carried out. As previously reported by Evans, Tsai and Young, the liver glycogen fell steadily, and the blood glucose first rose and then fell. The inorganic phosphorus showed an insignificant fall, but rose above the initial value again after 2 hours; the organic phosphorus after the first two samples fell considerably in one experiment, from 14 to 3 mg./100 c.c. in 1½ hours, but later rose again to 5·7. We have not further examined these delayed changes.

Relationship of adrenaline to the blood phosphorus in the decapitate cat. Barrenscheen, Eisler and Popper [1927] found in rabbits that as the

blood glucose rose after adrenaline injection the inorganic phosphorus of the blood was reduced. This has been confirmed by Cori and Cori [1932], who explained it as due to formation of hexose phosphate in muscle. We performed seven experiments on decapitate cats in which adrenaline was introduced slowly into the femoral vein at the time when recovery of liver glycogen had occurred after decapitation, and confirmed the observations of the workers mentioned. The curve of blood phosphorus was much the same as in the experiments in which decapitation alone was

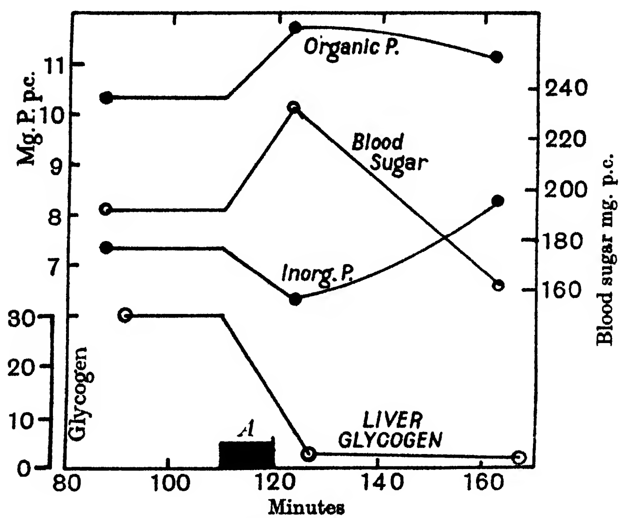

Fig. 2. Effect on blood phosphate and sugar and liver glycogen of infusion of 8 c.c. of 1 : 10,000 adrenaline into femoral vein of decapitated cat, during 10 min.

performed, *i.e.* there was a small fall in inorganic phosphorus and a corresponding rise in the organic phosphorus. The changes were quite small in amount, as shown in Fig. 2, which is illustrative of a typical experiment.

Effect of removal of both suprarenals on the blood phosphorus level. Removal of one suprarenal followed in 3 weeks by the removal of the other only caused a slight fall in the blood phosphorus as the following figures show:

	Inorganic phosphorus mg./100 c.c.	Organic phosphorus mg./100 c.c.
Cardiac puncture before operation	9·3	10·3
Cardiac puncture after removal of both suprarenals	8·6	9·4

Further work on adrenalectomized animals was not attempted because of their feeble resistance to experimental procedures.

Effect of the injection of phosphate and glucose on the storage of liver glycogen. Two fasted cats were decapitated in the usual way and after recovery of liver glycogen we gave an injection of glucose and disodium hydrogen phosphate, in order to see whether there would be a further laying down of liver glycogen. In one experiment 5 g. of glucose with 1 g. of Na_2HPO_4 in 50 c.c. of distilled water were injected slowly into the femoral vein, and in the other experiment the same composition and amount of solution was allowed to run slowly into the splenic vein. In both instances we failed to cause any considerable rise in liver glycogen, although in the second experiment the liver glycogen did reach a slightly higher level than the initial level just before decapitation. The inorganic phosphorus rose steadily after the infusion of the glucose phosphate mixture, but the organic phosphate was not appreciably altered. Introduction of glucose into the duodenum in another experiment failed also to cause any storage of liver glycogen, nor was there any change in the blood phosphorus level contrary to the findings of Barrenscheen *et al.* [1927], McCullagh [1931] and others, who noted an increase in both organic and inorganic phosphorus in the blood when glucose was given *per os*.

Summary.

Decapitation of cats, or injection of adrenaline after decapitation and recovery, caused a fall of about 1 mg./100 c.c. in the inorganic phosphate of the blood and a similar rise in the organic phosphate. These changes are so small as to give no grounds for supposing that any appreciable amounts of carbo-hydrate-phosphoric esters can enter the blood during decapitation, or that such compounds in the blood form a source from which liver glycogen is formed.

The expenses of this investigation have been in part borne out of Rockefeller Founda tion Funds (to T.V.), and in part out of a Grant from the Government Grants Committee of the Royal Society (to C.L.E.). Our best thanks are expressed for this assistance.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

IV. The effect of some anæsthetics.

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ERRATUM

Table I, Column A, 1st line

for 1·2 *read* 7·2

method. Prof. Shaffer has kindly furnished us (through Prof. Lovatt Evans) with the unpublished details of the modifications of the original procedure, and with his permission we herewith include these, as we find the method as now modified to be entirely satisfactory and to give theoretical results with pure glucose.

Modified Shaffer-Hartmann reagent.

Na_2CO_3	25 g. per litre
$NaHCO_3$	20 ,, ,, ,,
Rochelle Salt	25 ,, ,, ,,
$CuSO_4$	7·5 ,, ,, ,,
KIO_3	100 to 300 c.c. of a solution 0·1N as to I_2.

(We add 0·801 g. of KIO_3 per litre.)

5 c.c. of the neutralized glycogen hydrolysate are added to 5 c.c. of the above reagent and the mixture heated, in test-tubes (25 × 200 mm.) covered with glass bulbs, for 15 min. in a rapidly boiling water bath. After cooling in water to each is added 2 c.c. of a solution

[1] Bayliss-Starling Scholar. [2] Sharpey Scholar.

containing 2·5 p.c. of potassium iodide and 2·5 p.c. of potassium oxalate, and this is followed by 5 c.c. of $N H_2SO_4$. The tubes are kept covered by glass bulbs and, after shaking and standing for 5 to 10 min. to ensure complete solution and reoxidation of cuprous oxide and iodide, the mixture is titrated with 0·005 N thiosulphate. For calculating the results a curve may be constructed from the following data, supplied by Prof. Shaffer.

TABLE I.

Mg. glucose in 5 c.c.	Titration difference c.c. of 0·005 thiosulphate
2·00	18·20
1·75	15·95
1·00	9·00
0·50	4·48
0·10	0·85
0·05	0·42

Effect of sampling on glycogen content of lobes of the liver.

In accordance with the results described in the previous paper [Evans, Tsai and Young, 1931] the glycogen contents of different lobes of the liver have been considered to be comparable.

In order to determine whether the glycogen content of a lobe was significantly altered by the taking of samples, a number of simultaneous determinations were made on lobes, some of which had previously been sampled, and some of which had not, with the following results.

TABLE II. Glycogen content of used and unused liver lobes.

Exp.	Used lobe p.c.	Unused lobe p.c.
206	0·78	0·44
206	0·17	0·13
206	1·33	1·43
208	1·26	1·02
208	1·16	1·06
222	0·47	0·19
223	0·31	0·22
227	3·22	3·29
230	2·45	2·27
232	0·12	0·05
238	0·40	0·31
239	1·82	1·95
	Average 1·124	Average 1·030

Standard deviation = 0·087. Standard deviation = 0·094.

Unexpectedly it seems that the average of the used lobes is 0·094 p.c. greater than that of the unused lobes, *i.e.* nearly a 10 p.c. relative error.

Calculation of the standard deviation of the average for used and unused lobes suggests that this difference may or may not be significant, but whatever conclusion is drawn, it is certain that the glycogen content of used lobes is not lower, on the average, than that of unused lobes.

In a few cases one or more lobes appeared dark and bruised in consequence of handling or other injury. The glycogen content of such lobes was lower than that of the lobe which appeared quite normal, and histological examination showed that in the bruised sample there was stasis and congestion in the vessels and shrinkage and cytoplasmic cloudiness in the cells.

Blood sugar has been determined throughout by the method of Hagedorn and Jensen [1923]. Although Somogyi [1926] and others have shown that this method includes in the result what Benedict [1931] has termed "saccharoids," yet since we were interested principally in the relative blood glucose changes, we considered the method to be sufficient for our purpose.

At the beginning of each experiment we took a sample of normal blood from the ear for sugar determination. It was usually found possible to obtain such a sample without the slightest struggle while the cat was sitting peacefully on the table. In the few cases where struggling occurred the blood sugar level was always considerably above the average, and those results were not included in the average figures.

All the cats used in this investigation were fasted for 44–48 hours before the experiment.

Results.

Normal blood sugar of cats.

During the course of investigations in this laboratory blood samples from 89 unanæsthetized cats have been taken. The average blood sugar level of these animals was found to be 94·5 mg. p.c. (min. 62, max. 122).

In Table III are given the averages over various periods.

Table III. Average blood sugar for cats fasted 48 hours (Hagedorn and Jensen method).

Period	No. of cats	Blood sugar (mg. p.c.) Max.	Min.	Average
11. xi–10. xii. 31	22	120	79	98·8
11. xii. 31–26. i. 32	29	118	70	95·7
27. i–9. ii. 32	15	122	87	97·0
10. ii–25. iii. 32	23	121	62	86·9

During the period 9. ii–4. iii. 32, which included the coldest weather during the time of these experiments, the average blood sugar for 12 cats was 81·7 mg. p.c. The houses in which the animals are kept before experimentation are maintained thermostatically at a temperature of 60° F. The average duration of stay in the animal house is 4 days.

Whether the variation of average blood sugar for different periods shown in Table III is fortuitous, seasonal or dependent on temperature is, however, impossible to determine.

Effect of chloroform.

The induction of chloroform anæsthesia was effected by the use of an open mask, and continued after insertion of a tracheal T-cannula, by connecting this with a Woulffe's bottle containing chloroform, access of air being regulated by a screw clip on the side tube. In Fig. 1 are shown the average curves for liver glycogen and blood sugar obtained from three experiments. The liver in each case initially contained about 2 p.c. of glycogen, and the average curve has been extrapolated to 0 min.

It has been shown [Evans, Tsai and Young, 1931] that etherization for 50 min. reduces the liver glycogen to about 50 p.c. of its original level in cats. After 50 min. the liver glycogen content tended to rise slightly.

As was to be expected from its known toxic action on the liver, chloroform has a considerable effect on the glycogen content, reducing it to about one-quarter of the initial amount in 50 min. The blood sugar was also higher than that found under ether anæsthesia.

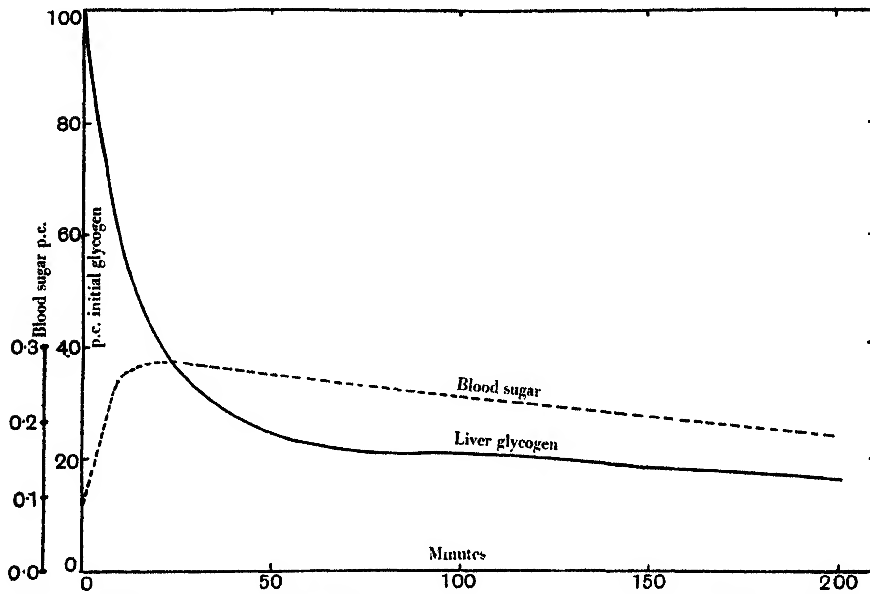

Fig. 1. Effect of chloroform anæsthesia on blood sugar and liver glycogen. Mean curves for three experiments. Chloroform begun at 0 min.

Effect of amytal.

Amytal (isoamyl ethyl barbituric acid) was one of the first anæsthetics shown to have little effect on the blood sugar level [Page, 1923; Edwards and Page, 1924], but in a previous paper [Evans, Tsai and Young, 1931] from this laboratory it was stated that amytal anæsthesia causes a considerable loss of liver glycogen in cats. This has been confirmed in the present investigation and the average blood sugar and liver glycogen curves for four experiments are given in Fig. 2.

We have found 100 mg. per kg. a generally satisfactory dose for intramuscular or intraperitoneal injection, but greater amounts (up to 160 mg. per kg.) are required subcutaneously.

Dann and Chambers [1932] have recently noted that the dose of amytal we used for cats (70–160 mg. per kg.) was considerably in excess of that required for dogs in their experiments (60 mg. per kg.), and consider that for dogs, at any rate, amytal anæsthesia is suitable for the study of carbohydrate metabolism.

We have invariably found with cats that if less than 70 mg. per kg. was injected intramuscularly satisfactory anæsthesia did not result even after 2 hours, at which time a further injection of 30 mg. per kg. produced surgical anæsthesia in 30 min. Our amytal was supplied by British Drug Houses, Ltd., and for injection was completely dissolved in 5–6 c.c. of dilute NaOH by warming to 50° C., the excess alkali being neutralized by dilute acetic acid, until the solution was faintly opalescent, one drop of alkali then being added to ensure complete solution.

Zerfas *et al.* [1928] find the intravenous anæsthetic dose for dogs to be 40–50 mg. per kg., whilst in man it is 20–25 mg. per kg., and they stress the fact that the *p*H of a 10 p.c. solution of the sodium salt of amytal in distilled water is 9·5–9·8, and claim that this *p*H gives a maximum anæsthetic effect in dogs with a minimum degree of toxicity. Lowering of the alkalinity of a 10 p.c. solution to *p*H 9·2–9·3, which causes some cloudiness, causes a striking loss in anæsthetic power, and a definite increase in toxicity.

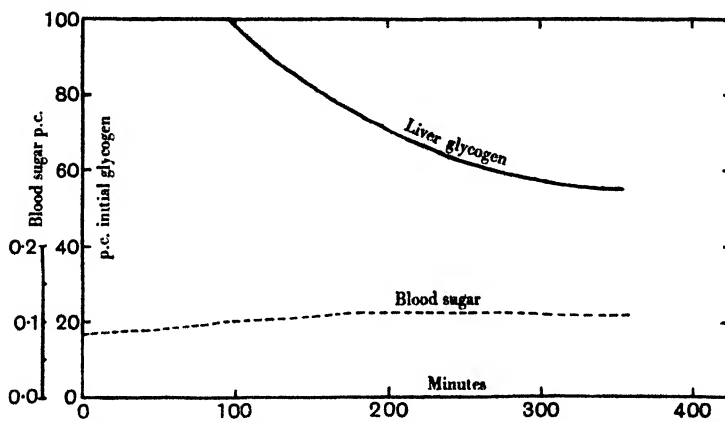

Fig. 2. Effect of amytal anæsthesia on liver glycogen and blood sugar. Mean of four experiments. Surgical anæsthesia about 100 min. after injection.

Page and Coryllos [1926] state that whereas 50 mg. per kg. intravenously is the anæsthetic dose for dogs, 15–20 mg. per kg. more is required for intraperitoneal injection. Fitch and Tatum [1932] find that the minimum lethal dose intraperitoneally for rabbits is 90 mg. per kg.

We are informed by Dr L. E. Bayliss (private communication) that he finds the amytal available in this laboratory to be less potent than that used by him at Harvard University (presumably Lilly's), of which he found 100 mg. per kg. to produce surgical anæsthesia in cats in 15 min. The amytal he used at Harvard was incompletely soluble in alkali, and after filtration was injected in alkaline solution, without neutralization.

We cannot explain this difference in potency of the amytal from these two sources.

Effect of dial.

Diallyl barbituric acid or dial (Society of Chemical Industry in Basle) was found to be effective in doses of 0·12 g. per kg. when dissolved in the same way as with amytal, surgical anæsthesia being induced in about an hour. The effect of dial in lowering liver glycogen and raising blood sugar

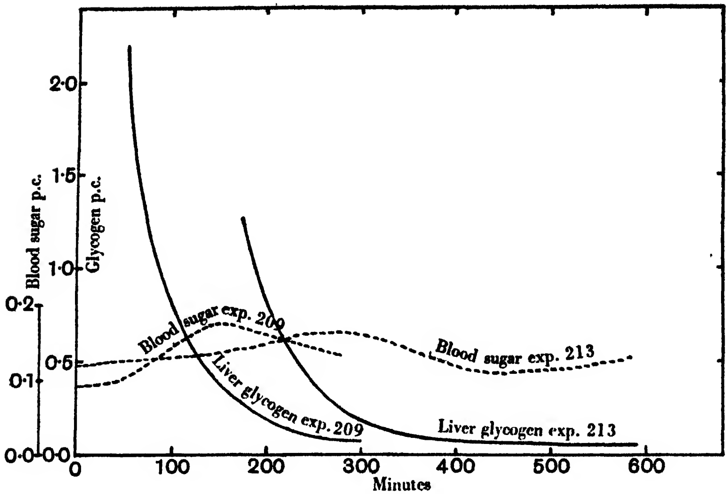

Fig. 3. The effect of dial anæsthesia on liver glycogen and blood sugar.

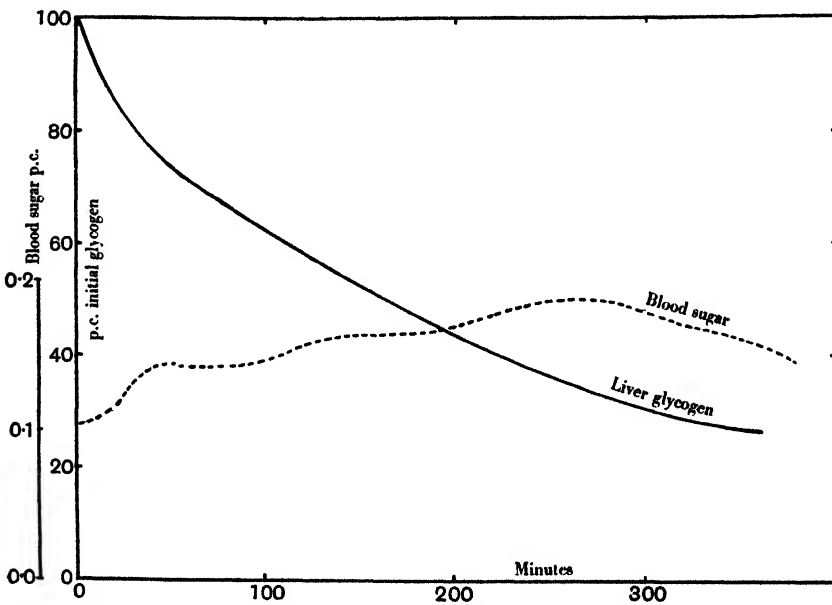

Fig. 4. Effect of luminal anæsthesia on liver glycogen and blood sugar. Mean of three experiments. Curve extrapolated to 100 p.c. liver glycogen at 0 min.

was found to be considerably greater than that of amytal, and the results are given in Fig. 3.

In spite of the fact that anæsthesia was very deep, the fall of liver glycogen content was extremely rapid, although the blood sugar was not raised above 175 mg.

Effect of luminal.

Sodium luminal (the sodium salt of phenyl ethyl barbituric acid (Bayer Products, Ltd.) in doses of 120–150 mg. per kg. in aqueous solution intraperitoneally was found to produce surgical anæsthesia in 20 min. In Fig. 4 are given the average liver glycogen and blood sugar curves for

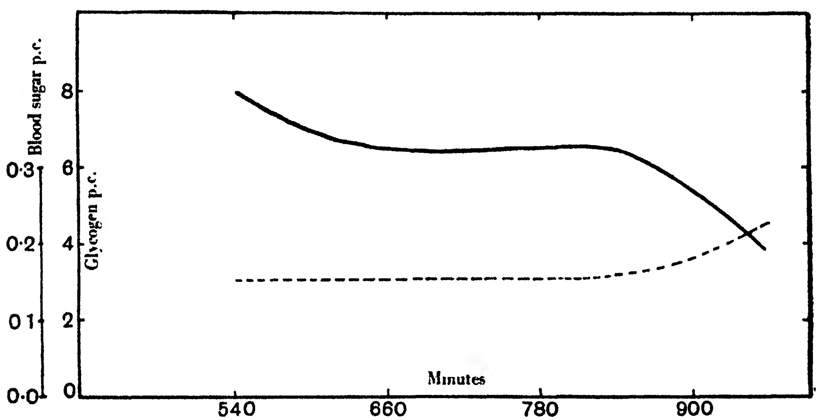

Fig. 5. Effect of luminal anæsthesia on liver glycogen and blood sugar after 9 hours of anæsthesia without liver sampling.

three experiments in which the initial liver sample was taken immediately after induction.

Arnell [1928] has stated that sodium luminal is without action on the sympathetic and the "parasympathetic" nervous systems. If this is so then it might be expected that if reflex effects were avoided this anæsthetic would have little effect on the glycogen stored in the liver.

This, however, was found not to be the case. The blood sugar progressively rose for 270 min. after which it fell, whilst the liver glycogen continuously diminished.

Figs. 5 and 6 give the results of two experiments in which the animal was allowed to remain anæsthetized during a long preliminary period before a liver sample was taken for analysis. By this means it was hoped that the liver glycogen would come to a steady state in which glycogenolysis was equal to glyconeogenesis.

During this preliminary period the temperature of the animal was carefully maintained at 37·5–38·5° C. on a hot plate, and water administered by a stomach tube if necessary. In one case blood sugar samples

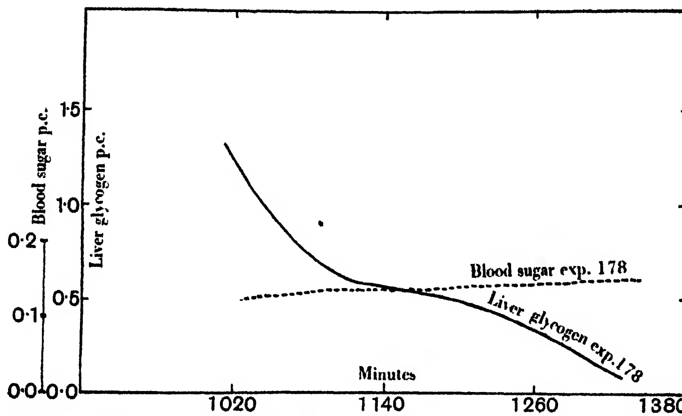

Fig. 6. Effect of luminal anæsthesia on liver glycogen and blood sugar, after 1000 min. of anæsthesia without liver sampling.

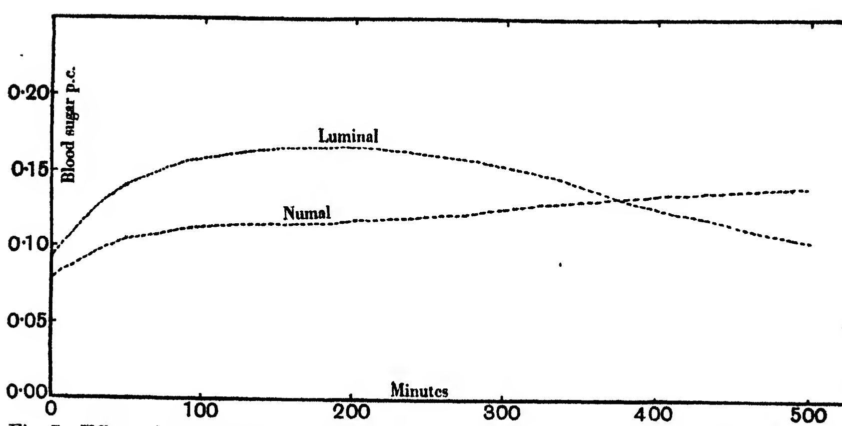

Fig. 7. Effect of luminal and numal anæsthesias on blood sugar. No liver samples taken. (Numal curve average of two experiments.)

were taken from the ear vein during this time, the curve being given in Fig. 7.

In the case of Fig. 5 the initial liver sample, which contained 8 p.c. glycogen, was taken after 9 hours of anæsthesia, and during the further period of 180 min., from 660 until 840 min., the liver glycogen and blood sugar showed little change.

In the experiment shown in Fig. 6, however, in which the initial liver sample was taken 17 hours after induction of anæsthesia, and contained 1·4 p.c. of glycogen, the liver glycogen content did not remain steady for any appreciable period, although from 1100 until 1220 min. the fall was very small.

Effect of numal.

Numal-Roche (the diallyl isopropryl barbiturate of diethylamine) is supplied by the makers in a 10 p.c. solution. Hoet and Ernould [1930] have found that this anæsthetic does not affect the blood sugar in rabbits,

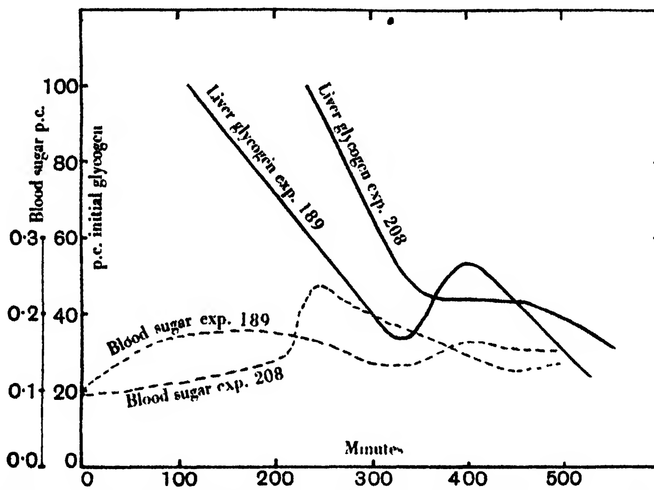

Fig. 8. Effect of numal anæsthesia on blood sugar and liver glycogen. Dose 0·7 c.c. per kg. intraperitoneally. Glycogen content of first sample in both cases approximately 3 p.c.

but Clark [1931] found that during surgical anæsthesia by numal in rabbits, and probably in cats, the hyperglycæmia following the intravenous injection of glucose is prolonged.

We have found (Fig. 7) that in an experiment in which blood samples were taken at intervals from the ear veins of two cats before and during induction of anæsthesia with numal, the average blood sugar steadily rose from 80 to 150 mg. in 500 min.

We have found the intraperitoneal injection of 0·5–0·8 c.c. per kg. of numal to be effective in producing surgical anæsthesia in about 100 min. in cats.

In Fig. 8 are given the results of experiments in which the liver samplings extended from 100 to 500 min. after the injection of numal,

whilst in Fig. 9 are given the results of two experiments in which a preliminary period of about 10 hours of anæsthesia preceded the liver sampling. In another experiment (Fig. 10) the first liver sample was

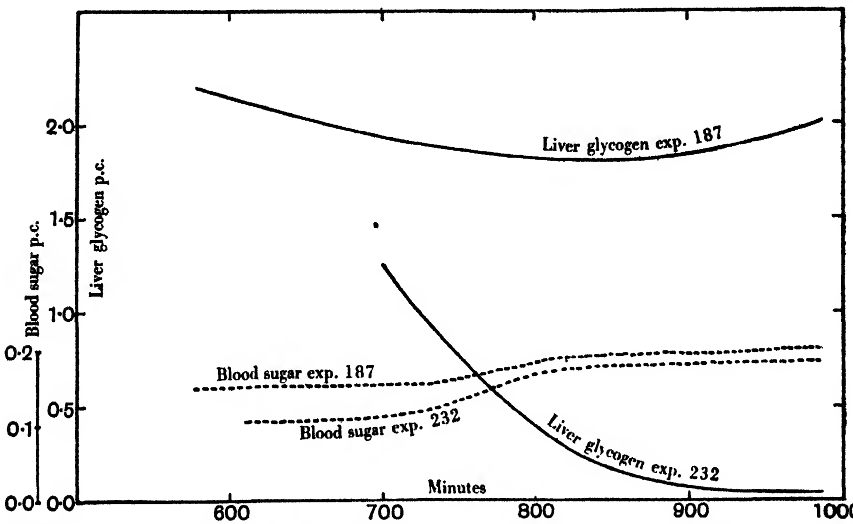

Fig. 9. The effect of numal anæsthesia on blood sugar and liver glycogen: 600–700 min. of anæsthesia previous to liver sampling.

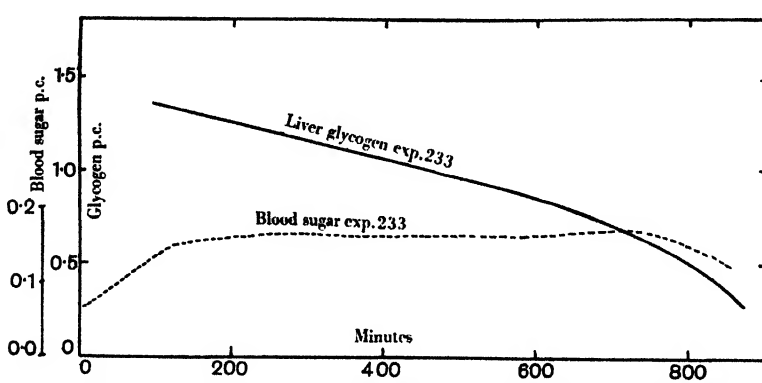

Fig. 10. Effect of numal anæsthesia on blood sugar and liver glycogen. First liver sample taken immediately after induction, the second 10 hours later.

taken immediately after anæsthesia had become satisfactory, the next being taken 10 hours after.

It seems from these results that the effect of numal anæsthesia on liver glycogen is very variable, though in all cases except one there was a

decided fall in the liver glycogen content, but it is difficult to explain the discrepancy between the glycogen curves shown in Fig. 9, as these two experiments were performed under similar conditions.

Effect of pernocton.

Pernocton is the sodium salt of sec. butyl bromallyl barbituric acid and is supplied by its makers (J. D. Riedel, Berlin) in 10 p.c. solution. The dose recommended is 1 c.c. per $12\frac{1}{2}$–15 kg. body weight, but we found 0·5 c.c. injected intraperitoneally did not anæsthetize a cat weighing 2 kg. in 2 hours, after which time a further injection of 1 c.c. produced

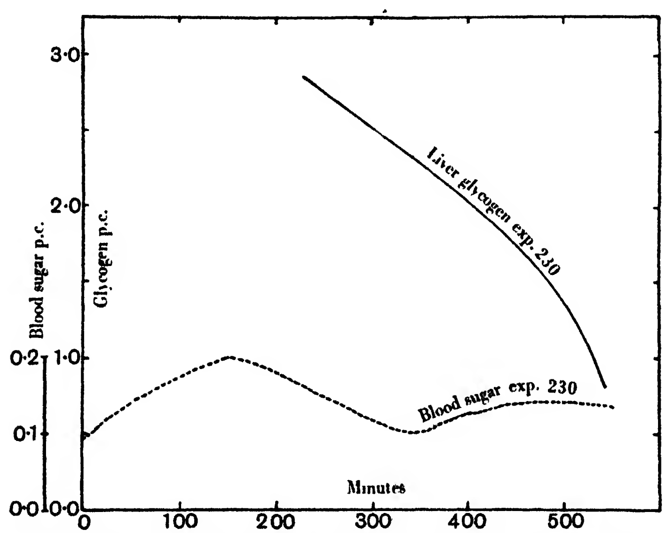

Fig. 11. The effect of pernocton anæsthesia on liver glycogen and blood sugar.

surgical anæsthesia after 1 hour. The effect on liver glycogen and blood sugar is shown in Fig. 11.

According to Dimitrijević [1930], in dogs the margin between surgical anæsthesia and death is narrow, and small rises of blood sugar occur under pernocton anæsthesia, though Matakas [1931] finds blood sugar and lactate are unaffected.

Effect of chloralose.

Vincent and Thompson [1928] found that chloralose raised the blood-pressure of cats and suggested that the secretion of adrenaline had been stimulated, but Tournade and Hermann [1928] could find no evidence for this theory.

We have found, in confirmation of Griffith [1923], that chloralose affects the blood sugar level very little, the theory of increased adrenaline

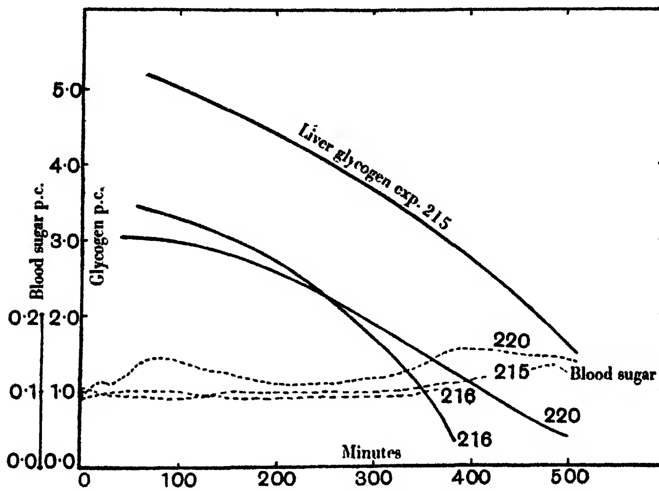

Fig. 12. Effect of chloralose anæsthesia on blood sugar and liver glycogen.

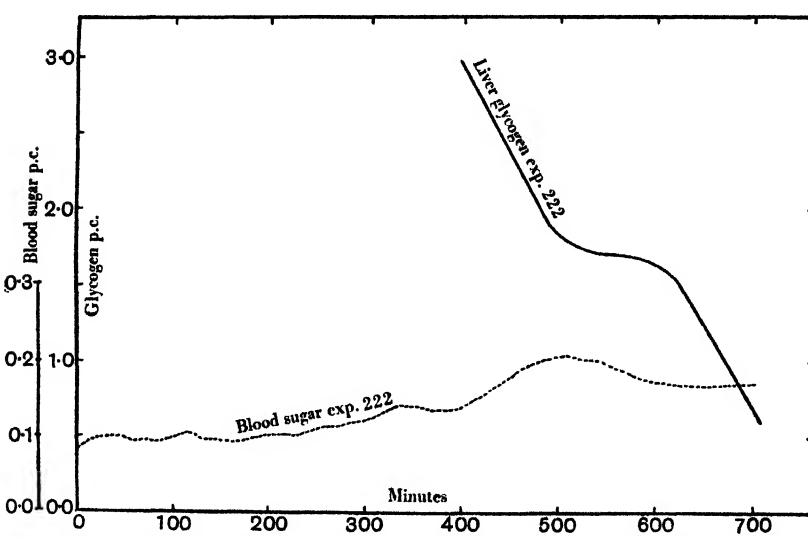

Fig. 13. Effect of chloralose anæsthesia on blood sugar and liver glycogen: $6\frac{1}{2}$ hours of anæsthesia previous to liver sampling.

stimulation being thus not substantiated. Nevertheless, under the anæsthesia produced by subcutaneous injection of 0·1 g. per kg. body weight,

it was found, as it was by Bodo and Neuwirth [1931] in dogs, that the liver of cats rapidly lost glycogen (Fig. 12).

In Fig. 13 is given the result of one experiment in which a preliminary period of blood samples under chloralose anæsthesia preceded the taking of liver samples. In this cat the fall of glycogen was rapid at first followed by a period (500–620 min.) during which there was little fall, and again succeeded by another rapid fall. On the whole, however, the glycogen disappeared from the liver more quickly than in the short period experiments with chloralose.

In view of the very small change in blood sugar of cats anæsthetized with chloralose, the increasing rate of disappearance of liver glycogen with increasing time is rather surprising.

Magenta [1927] finds that chloralose causes a rise of blood sugar in the first 15 min., this being followed by a fall for 30–60 min. We found that the effect on the blood sugar was rather a slight fall at first with a slight rise after 5 hours. This eliminates the possibility that chloralose exerts its narcotic effect by the liberation of small quantities of either chloral or chloroform, as both of these anæsthetics produce a considerable hyperglycæmia [Steinmetzer and Swoboda, 1928].

Nembutal.

It has been suggested that nembutal (E. H. Spicer and Co.) has less effect on the liver than other anæsthetics used clinically, and we have carried out one experiment with this. As with other anæsthetics the liver glycogen fell continuously while the blood sugar rose somewhat.

DISCUSSION.

Of the various anæsthetics we have tried, the blood sugar was least affected by chloralose, and, of the barbituric acid derivatives, least by amytal.

It is obvious that no anæsthetic that we have tried leaves the carbohydrate metabolism of the body undisturbed, under the conditions of our experiment, which involved the taking of successive samples of the liver. It would in fact appear very unlikely that any method of anæsthetization would eliminate the reflex glycogenolysis consequent upon operative procedures, without exerting some direct toxic action on the liver instead.

It seems, on the evidence of extrapolation, which is only approximate in view of the long period between induction and commencement of sampling, that the fall of liver glycogen, which is probably continuous from induction, is accelerated by the process of sampling.

It was also clear from experiments in which blood sugar determinations were made during a preliminary period of anæsthesia that the blood level was raised somewhat as soon as sampling of the liver commenced.

The rise in blood lactate and blood glucose, and the fall in alkali reserve [Fuss, 1931] under ether anæsthesia, together with the fall in liver glycogen, can in part be attributed to asphyxia, to a direct action of the anæsthetic on the liver, and to the liberation of adrenaline from the suprarenals [Macleod, 1926; Evans, Tsai and Young, *loc. cit.*]. Chloroform would appear to have a still more potent action of the same nature.

Brown and Garry [quoted by Clark, 1931] find that chloralose has less effect on the autonomic nervous system than any other anæsthetic they have tried. In experiments with cats under chloralose, Clark [1931] denervated the liver and tied off the suprarenals: he then found that there was a steady fall in blood sugar which was accelerated for a short time by the cutting of the right vagus. If his interpretation of this, *i.e.* that the vagus carries inhibitory fibres to the islets of Langerhans, be accepted, it seems possible from our experiments that chloralose may decrease the action of these inhibitory fibres, thus resulting in a liberation of insulin.

In those experiments in which the animal was kept under luminal or chloralose anæsthesia for a considerable period before the first liver sample was taken, there was, as shown by the curves (Figs. 5 and 13), a period during which the fall of liver glycogen was negligible.

During this period glycogenolysis was apparently either suppressed completely, or else was equal to the glycogen formation from protein fat or lactate: formation of glycogen from glucose is ruled out, in the case of luminal at least, by consideration of the blood sugar curves.

With numal anæsthesia in two cases, Fig. 8, Exp. 189, and Fig. 9, Exp. 187, the liver glycogen actually began to rise after the preliminary fall, the rise in each case being accompanied by a slight rise in blood sugar. It is clearly evident that glycogen formation could not have occurred from blood glucose, as this was rising, so that it must have taken place from some other source, possibly by glyconeogenesis, although evidence considered later tends to the view that amytal anæsthesia inhibits glyconeogenesis.

In most cases it is clearly impossible to account for the disappearance of liver glycogen by an increase in blood sugar. This is most clearly

seen in the action of chloralose, as the following detailed protocol shows:

Cat. ♀. 2·10 kg. Fasted 44 hours.

Time		Blood glucose p.c.	Liver glycogen p.c. (mean of two)
11.05	Blood sample from ear	0·101	—
11.06	Chloralose, 0·12 g./kg. intraperitoneally	—	—
11.27	Blood sample	0·098	—
11.58	Liver samples	—	3·48
11.59	Blood sample	0·104	—
3.07	Blood sample	0·097	—
3.14	Liver samples	—	2·21
5.00	Blood sample	0·111	—
5.01	Liver samples	—	0·88
5.50	Blood sample	0·113	—
5.52	Liver samples	—	0·19

Assuming that the blood sugar is in equilibrium with a volume of blood *plus* tissue fluids together equivalent to one-third of the body weight, the cat used in this experiment had 700 c.c. of fluid available for diffusion. At the beginning of the experiment the liver weighed 47 g., so that a fall of liver glycogen content from 3·48 p.c. at 11.58 a.m. to 2·21 p.c. at 3.14 p.m. should cause a rise of blood sugar of 0·085 p.c., *i.e.* glucose was being removed at the rate of 0·026 p.c. per hour (= 0·18 g. per hour).

In three experiments in which muscle and liver glycogens were determined at 60 and 350 min. after chloralose injection it was found that on the average the fall of liver glycogen during the period 100–350 min. was 0·65 p.c., whilst the muscle glycogen fell by 0·04 p.c.

The livers were weighed in each case, and the average loss of liver glycogen was calculated to be 0·46 g. Assuming that the musculature of the animal was equal in weight to one-half of the total weight of the animal the fall in muscle glycogen accounted for the disappearance of 0·93 g. of glycogen. During this period the blood sugar rose by an average of 20 mg. p.c., whilst the blood lactate fell by an average of 5 mg. p.c.

If the assumption is made that the volume of fluid in equilibrium with the blood sugar and lactate is equal to one-third of the body weight [Evans, Tsai and Young, 1931], then the rise of blood sugar and fall of lactate can together account for the disappearance of 0·17 g. of glycogen from the liver and muscles.

Thus of the 1·39 g. of glycogen which disappears from the glycogen stores of the body, only 0·17 g. can be accounted for in the blood, leaving a balance of 1·22 g. A similar result is obtained for experiments under amytal; presumably the 1·22 g. of glucose disappearing in 4 hours can be

more than accounted for by combustion in our experiments [Best, Dale, Hoet and Marks, 1926].

The question that remains, however, is, why do the glycogen stores of the anæsthetized cat suffer this depletion during a period that leaves the glycogen of an unanæsthetized cat materially unaffected?

The basal metabolic rate of amytalized animals differs little from the normal [Lee, 1928; Deuel, Chambers and Milhorat, 1926], and similar results would be expected for other anæsthetics, so that increased metabolism would not account for this difference.

The most reasonable assumption would seem to be that in the normal unanæsthetized animal the liver is continuously manufacturing glycogen, and that this "secretion" is inhibited by anæsthesia.

That amytal inhibits experimental hyperglycæmia has been confirmed by Olmsted and Giragossintz [1931] in the case of hyperglycæmia due to morphia and to asphyxia, and by Donhoffer and Macleod [1932] in the case of that due to asphyxia; Olmsted and Giragossintz [1931] suggest that amytal anæsthesia tends to inhibit glycogenolysis.

Donhoffer and Macleod [1932] have suggested that in the fasting rabbit decerebrated through the pons and with little liver glycogen, hyperglycæmia is due to stimulation of the glyconeogenic process in the liver by way of the parasympathetic nerves, and that this glyconeogenesis is inhibited by administration of atropine with section of both vagus nerves, and by amytal.

In view of recent evidence suggesting that amytal inhibits the parasympathetic [Weiss, 1929; Leib and Mulinos, 1929; Shafer, Underwood and Gaynor, 1930; Garry, 1930; Olmsted and Giragossintz, 1930; Donhoffer and Macleod, 1932] it would seem possible that the fall of liver glycogen under amytal anæsthesia is due to inhibition of the parasympathetic, and consequently of glyconeogenesis. Whether such a statement is true for other anæsthetics, in particular, for chloralose, cannot be decided on the available evidence.

However, as all anæsthetics that we have tried cause a decrease of liver glycogen under the conditions of our experiments, we must draw the conclusion that such conditions are unsuitable for the sudy of carbohydrate metabolism of the normal unanæsthetized animal.

SUMMARY.

1. The effect of anæsthesia by chloroform, amytal, luminal, dial, numal, pernocton, chloralose on blood sugar and liver glycogen of the cat has been investigated.

2. In all the experiments in which liver samples were removed at intervals for analysis, it was found that the liver glycogen content fell throughout, or at some time.

3. Of the anæsthetics tried, on the average there was least fall of glycogen with amytal, whilst chloralose had least effect on the blood sugar.

4. In experiments in which the animal was anæsthetized some hours before the first liver sample was taken, the liver glycogen curve showed a "plateau," for the duration of which the fall of liver glycogen was small.

5. This can be considered to be evidence of the formation of glycogen in the liver, from non-carbohydrate sources.

6. It is concluded that experiments carried out under the above anæsthetics involving the taking of liver samples from experimental animals are unsatisfactory.

We wish to express our thanks to Prof. C. Lovatt Evans for much helpful advice and criticism, and to Mr C. A. N. Evans for technical assistance. We are also indebted to the Clayton Aniline Co. for samples of dial, to the Hoffman-la-Roche Co. for supplies of numal, and to Messrs Spicer for Nembutal.

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THE BEHAVIOUR OF LIVER GLYCOGEN IN EXPERIMENTAL ANIMALS.

V. Some factors affecting liver glycogen recovery in the decapitate cat.

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Introduction.

The observation by Olmsted and Coulthard [1928] that the liver glycogen of the cat, lowered by decapitation, subsequently shows considerable recovery, was further investigated by Evans, Tsai and Young [1931 *b*] who pointed out that the decapitate animal during this recovery exhibits a rate of glycogen formation not to be obtained in any other laboratory preparation.

In continuance of that work we have further investigated some of the factors which may play a part in causing the restoration of liver glycogen in the decapitate cat.

The present paper deals principally with further consideration of the methods used, and with the influence of alterations in the amount and composition of blood supplied to the liver.

Methods.

The methods used were those described in the previous papers of Evans, Tsai and Young, and Murphy and Young [1932] with the following minor alterations:

(1) In order to ensure proper artificial respiration, the cats were ventilated, from just prior to decapitation, with a known volume of air (600–1000 c.c. per min., according to the weight of the animal). The double-action Schuster [1922] pump was generally used, but the Starling Ideal [1926] or the Olmsted and Taylor pump were employed on occasion.

(2) Carotid blood-pressures were taken at the times of blood sampling, and were usually found to remain constant at 50–80 mm. Hg, for several hours after decapitation. The fall to below 50 mm. after about 300 min. was often accompanied by a fall in liver glycogen. The average of 30 experiments showed that this deterioration began after the removal of 4·3 duplicate samples, at 260 min. after ether induction, and was sometimes accompanied by a slight terminal rise in blood sugar level.

[1] Bayliss-Starling Scholar. [2] Sharpey Scholar.

The procedure in the experiments was usually as follows: The cats were fasted for 44–48 hours. After taking a blood sample from an ear vein for blood glucose estimations, ether was given by an open mask, and all times were measured from the beginning of ether anæsthesia. Usual times for the ensuing procedures were:

0 min. Ether anæsthesia commenced.

4–6 min. Duplicate liver samples for glycogen. Tracheal cannulation.

12 min. Both carotids tied. Carotid blood sample.

17–25 min. Decapitation complete. Artificial respiration on. Ether off.

Then carotid samples and liver samples having been taken and blood-pressure records started, the experimental manipulation proper to the particular experiment was begun, and at suitable intervals subsequently carotid blood-pressure was noted, and blood and liver samples were taken.

After each removal of liver samples any scraps were also weighed, and at the end of the experiment a post-mortem examination of the carcase was made and the remaining liver weighed. The weight of the liver at each stage of the experiment could then be computed.

I. Further considerations regarding the methods.

(1) *Water content of liver and blood.*

It was just conceivable that the increase in liver glycogen in our preparations might be in part due to a loss of water from the liver. A number of liver samples were accordingly taken at various stages of the experiment, and dried to constant weight at 110° C. The moisture content estimated thus was found to be 69 p.c. $\pm$ 1 p.c., and under the conditions of the average experiment was found to be constant. This is in agreement with Bridge and Bridges [1931], who find in a series of rabbit livers with widely varying glycogen contents, a water content of 72 $\pm$ 4 p.c. Puckett and Wiley [1932] and MacKay and Bergman [1932] give similar values. We see no reason to differ from the conclusions of these various authors, or to suppose that in acute experiments such as our own the glycogen content is appreciably affected by water changes. The hæmoglobin content of the blood was also reasonably constant during the time of the experiments.

(2) *Fat content of the liver.*

In some preliminary experiments liver fat was estimated in successive samples, using the technique of Hynd and Rotter [1930], with a view to testing the theory, originally advanced by Rosenfeld [1903], of the reciprocal contents of fat and glycogen in the liver. In one experiment, shown below, there was a slight increase in "fat" (calculated from the fatty acid values with assumed mean molecular weight of linoleic acid – 280), but as there was no indication of any reciprocal adjustment between liver fat and glycogen contents in these experiments the subject was not further pursued.

Exp. 227. Cat. 2·25 kg. Fasted 44 hours.

	Liver		
Min.	Glycogen p.c.	Fat p.c.	Moisture p.c.
5	4·50	7·61	68·0
29 Decapitation	—	—	—
42	1·86	8·42	69·7
147	3·22	8·70	69·5

(3) *Method of liver sampling.*

The extended experience which has been gained with the methods employed for liver sampling has shown some important features which bear upon the interpretation of the results.

The experimental errors in the actual determination of glycogen in a given pair of duplicate samples are small and are chiefly due to the slightly greater degree of glycogenolysis in the second sample as compared with the first, and to experimental errors involved in the subsequent estimation. On the average these two sources of error only affect the results to the extent of 0·1 p.c. of glycogen.

The taking of two pairs of samples, at intervals, from the same lobe of the liver also does not result in the second samples giving too low values as compared with fresh lobes; indeed, as Murphy and Young [1932] have shown, the average for the second sample is usually very slightly raised as compared with intact lobes.

A more real source of error is the occasional variation in glycogen content from lobe to lobe, for although, as previously pointed out by Evans, Tsai and Young [1931*a*], on the average the lobes contain equal percentages of glycogen, still the occasional variations which are encountered make it important not to place too much reliance on the results of individual experiments. As would be expected, especially fluctuating results, with maintained high blood sugar and poor recovery of glycogen after decapitation, are liable to be encountered in those experiments which involve much manipulation of the abdominal viscera, including the liver itself: such manipulation or cooling of the viscera must therefore be reduced to a minimum.

It is our practice to weigh all the liver tissue removed at each stage, since the amount of liver remaining intact is of importance for two reasons. First, the removal of too much liver tissue might lead to portal obstruction, and this is particularly likely to happen if the lobes are sampled too far from their margins. The intestines were always carefully observed to ensure that there was no congestion. Secondly, as the liver is reduced by the taking of successive samples, an increase in its glycogen content becomes less and less significant to the balance-sheet of the whole body. An examination of 15 successive experiments taken at random showed that the livers had at the commencement of the experiments an average weight of 73·2 g. (2·8 p.c. of the body weight): the average weight of each sample (duplicate) removed, together with waste, was 2·48 g., *i.e.* in the taking of an average of nearly six samples some 14·7 g. of liver tissue (20·2 p.c. of the weight of the liver) were actually cut away.

But, in fact, more than this amount was put out of circulation by being enclosed between the bamboo strips or tapes used to check hæmorrhage; this amount varies with the number of lobes sampled and so with the number of strips or tapes simultaneously present on the liver. Estimating the tissue thus excluded, in the 15 experiments quoted above, the average loss by exclusion from the circulation of functional liver tissue amounts to about 3·3 g. per sample taken (2·43 min., 3·89 max.), which would mean that after six samples 19·8 g. of liver (27 p.c. of its weight) were out of commission. We do not consider it safe to go beyond this amount.

Now, supposing we start with a liver weighing 75 g. and containing 1·65 p.c. glycogen at the first sample, 0·66 p.c. after decapitation, 1·1 p.c. at the 100th minute and 1·58 p.c. at 300 min. (as in Evans, Tsai and Young's averages), and calculate the actual glycogen contents, with and without allowance for the reduction of liver tissue by three samples removing 3·3 g. each:

	Liver glycogen p.c.	Reduction of tissue: Not allowed for		Reduction of tissue: Allowed for	
After 1st sample	1·65	1·240	=0·745 g. loss	1·185	=0·735 g. loss
,, 2nd ,,	0·66	0·495		0·450	
,, 3rd ,,	1·10	0·820	=0·325 g. gain	0·710	=0·260 g. gain
,, 4th ,,	1·58	1·180	=0·360 g. gain	0·970	=0·260 g. gain
		Total gain = 0·685 g.		Total gain = 0·520 g.	

Similarly, whereas Evans, Tsai and Young [1931*b*, p. 94] calculate that between the 100th and 300th min. a 70 g. liver has gained 0·34 g. glycogen, recalculation shows that if two samples were taken, one at each of those times, the actual gain would be 0·32 g. The error due to loss by sampling does not appreciably affect their actual argument, but, as shown above, it becomes considerable when several successive samplings are considered. It will be evident that losses of total liver glycogen to the remainder of the body are also apparently increased, though to a smaller extent, by successive sampling.

(4) *Mode of expressing liver glycogen changes.*

The method used by Evans, Tsai and Young for expressing their results was to take a first sample under ether, and then calculate from this what was the approximate initial glycogen percentage before ether was given. This procedure involved the assumption that the livers of different animals lost the same fraction of their glycogen in the same time, while under ether; but it can be shown that if the animal has not been for many minutes under ether, the error is not likely to be great. The glycogen

contents throughout the experiment were then expressed as a percentage of this initial value. It was clear that this method left much to be desired, since the actual glycogen percentages in the livers of different 44-hour fasted cats varied greatly (from 0·33 to 10·0 p.c. in our joint experience), so that a loss or gain of a given percentage of the initial glycogen might represent widely differing amounts of glycogen. Careful inspection of all our results revealed the fact that often, though not invariably, the most rapid recovery was apparent in those experiments in which the initial

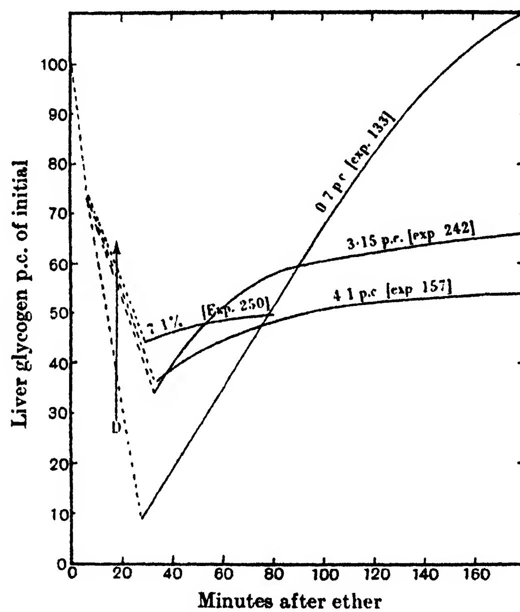

Fig. 1. Graph showing fall and recovery of liver glycogen of decapitate cats. Decapitation at the arrow marked *D*. Above each curve is shown the initial liver glycogen content.

concentration of glycogen was low, and in those experiments, moreover, a greater fraction of the liver glycogen was lost on decapitation. These facts are illustrated by Fig. 1 which gives the curve for four experiments taken at random and expressed in the manner described above.

It is clear from this example, as from most of our results, that the livers with the lower glycogen contents have lost a greater fraction of their glycogen on decapitation and have regained a still greater proportion of it on recovery: this is shown clearly by examination of the slopes of the recovery curves. Further examination shows that there was often a tendency for livers of similar size to recover total weights of glycogen of

about the same order during the same time in the early periods of the recovery. The weights of glycogen recovered were, however, not sufficiently near together to make it worth while expressing our results in that form for most of our purposes. We shall therefore adhere to the method previously employed, though it is important to bear in mind that the results of individual experiments cannot be properly compared with one another unless, among other identical conditions, their initial glycogen contents are similar.

It is perhaps not without significance in the present connection that Donhoffer and Macleod [1932] found with rabbits that after decerebration at the pons the liver glycogen was usually reduced, but that if the initial glycogen was below 0·7 p.c., there was an increase after decerebration; it was clear that some factor favouring recovery was present in those cases where the initial glycogen was low. Their experience, and our own, make it not unlikely that the factor in question is one which accelerates glyconeogenesis.

II. (1) Effect of alteration of the blood supply to the liver.

The two main supplies of blood to the liver evidently subserve different functions, since ligation of branches of the hepatic artery leads to necrosis, while the occlusion of branches of the portal vein leads to atrophy [Bainbridge and Leathes, 1907]. Since it is a common practice in the performance of perfusions of the liver to circulate the blood only through the portal vein, it seemed not unlikely that the failure of those preparations to store glycogen might have been due to the omission of the arterial circulation.

(a) *Ligation of the hepatic artery.*

Somewhat different estimates have been given by various investigators for the relative contributions to the hepatic blood supply of the hepatic artery and portal vein. According to Burton-Opitz [1911], and Macleod and Pearce [1914], about 30 p.c. of the total flow through the liver, in the dog, is derived from its arterial supply. Barcroft and Shore [1912], for the cat, give figures ranging from 13 to 58 p.c., the higher figure being for fed animals: they believed that most of the oxygen supply is obtained from the hepatic artery blood. Grab, Janssen and Rein [1929], for the dog, give the smaller figure of about 18–25 p.c. for the arterial fraction, while Bauer, Dale, Poulsson and Richards

[1932] in artificial perfusion found 25–30 p.c. for cat and dog. It would seem certain, from these results, that at least one-quarter of the blood supply to the liver is conveyed by the hepatic arteries.

It is well established that, in man, complete occlusion of the hepatic artery supply is not incompatible with life. In other animals too, many instances are recorded of recovery after ligation of the hepatic artery; an analysis of such cases is given in the papers by Segall [1923] and Cameron and Mayes [1930]. The explanation of these results is that ligation of the hepatic artery, even if complete, does not necessarily connote total deprivation of arterial blood supply to the liver, since, as pointed out by Haberer [1906], Segall [1923] and others, there are anastomotic connections of variable extent between the branches of the hepatic artery and those of the diaphragmatic arteries.

In the cat, the effects of complete occlusion of the hepatic artery are usually severe, though exceptions attributable to adequate collateral circulation were found by Haberer.

It has been clearly shown that after complete deprivation of its arterial blood supply the liver undergoes necrotic changes [Bainbridge and Leathes, 1907; Cameron and Mayes, 1930; and others], at an early stage of which its glycogen is lost. The general importance of the hepatic artery supply in carbohydrate metabolism was indicated by Collens [1925]. Collens, Shelling and Byron [1926, 1927] further found that ligation of the hepatic artery in dogs often caused death in 15–50 hours in hypoglycæmic convulsions, with complete loss of glycogen from the liver, heart and muscles. The period of survival after ligation they found to depend upon the glycogen content of the body. Winternitz [1911] and others describe the pathological effects as being different for different animals, and obtain as a rule little effect on the dog. It is probable that some earlier workers, not quoted here, did not get complete deprivation of arterial supply in their experiments.

In view of the facts just mentioned, it might be expected that on ligation of the hepatic artery in the decapitate cat there would be a failure of the normal recovery of glycogen if the latter was in any way dependent, as the ultimate survival of the liver tissue is dependent, on the provision of a proper supply of arterial blood. As will be shown, this expectation was not realized, for we found the recovery to occur fairly well in spite of the severity of the operative handling.

The arterial supply was first studied in injected preparations, from one of which Fig. 2 was sketched. There are considerable individual variations.

The hepatic arterial supply was cut off by two ligatures applied, one between the right gastric artery and the root of the hepatic artery proper (*b*, Fig. 2), and one to the gastro-duodenal artery (*a*, Fig. 2). This is necessary because the hepatic artery, after giving off the gastro-duodenal, is almost invariably split up into three or four lobar branches, the

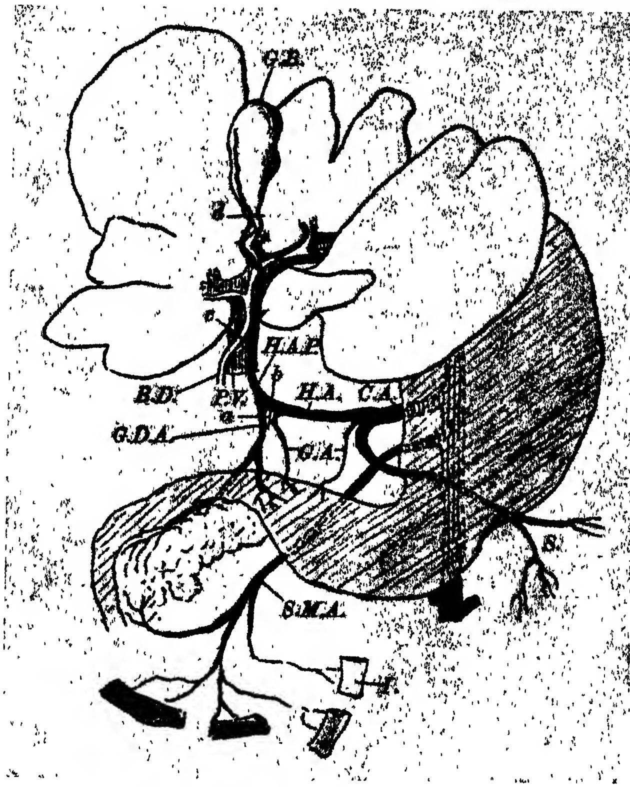

Fig. 2. Arterial supply to the cat's liver. *C.A.* cœliac axis; *G.A.* gastric arteries; *H.A.* hepatic artery; *H.A.P.* hepatic artery proper; *S.* splenic artery; *G.D.A.* gastroduodenal artery; *S.M.A.* superior mesenteric artery; *B.D.* bile duct; *P.V.* portal vein; *a* and *b*, position of ligatures on arteries; *c* and *d*, ligatures on bile ducts.

posterior of which are inaccessible for ligation as they pass medially and backwards to the left and behind the portal vein, which has the bile duct lying above its right border; further, because the collateral circulation by the gastro-duodenal artery is known to be adequate to maintain the liver. The effectiveness of the ligation was confirmed at post-mortem. It was complete in three experiments and incomplete in one.

The visible effects of the ligation during the course of the experiments, which lasted up to 7 hours, were not very striking—the liver became darker in colour, and showed congested patches, while the gall bladder, though not distended, was generally much lighter in colour than the normal, a greenish yellow in the most extreme cases—and there was some degree of diffusion of bile from the gall bladder on to adjacent parts of the liver.

In one experiment the animal was killed 6½ hours after the ligation. Some portions of the liver appeared very congested in patches, with appearance of early necrosis; the greater part of the tissue appeared to be only a little more solid than the normal. Portions fixed in Bouin's fluid confirmed the naked eye appearance. The normal-looking portion had a

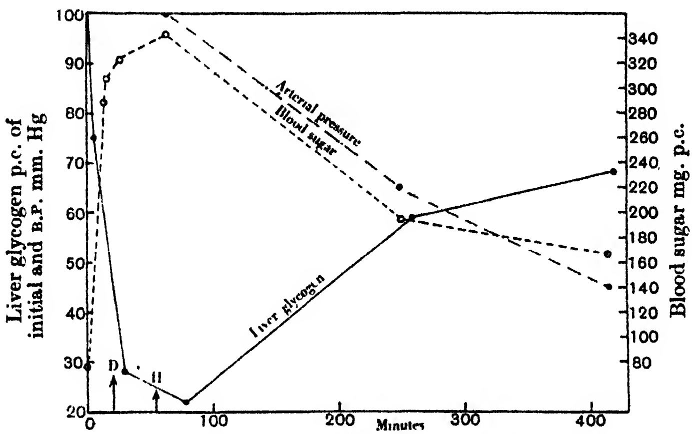

Fig. 3. Cat. Calculated initial liver glycogen concentration = 5·8 p.c. Decapitation at *D*. Glycogen at 30 min. = 1·62 p.c. Hepatic arteries ligatured at *H*. Glycogen at 79 min. = 1·29 p.c.: at 259 min. = 3·44 p.c.: at 420 min. = 3·94 p.c.

normal structure, the congested portion showed much the same appearance as described by Cameron and Mayes. There was great engorgement of all the sinusoids and of the branches of the portal vein and hepatic artery. In the portal vein blood were many mononuclear cells, looking like large detached Kupffer cells. The cytoplasm of the cells was much more cloudy than in the normal patches and showed vacuolation in some areas near to the intralobular veins.

The result of one of the experiments with complete ligation is shown in Fig. 3. The other three gave similar results, though in one of them the rise, which was good up to about 200 min. was then replaced by a fall.

In two experiments the blood sugar reached a high level after the ligation of the arteries (0·333 and 0·343 p.c.), and in three experiments out of the four, fell rather less quickly than usual, and even at about 350 min. still had, for the four experiments, a mean value of 0·122 p.c., whereas in normal conditions the amount would be below 0·1 p.c.

It appears from these results that, whatever may be the function of the arterial supply to the liver, its presence is not a basal necessity for glycogen formation, since it can be dispensed with for several hours.

In attempting to interpret this unexpected result some reference is necessary to the current views regarding the relationship between hepatic and portal circulations, an adequate summary of which is given by Cameron and Mayes [1930], Olds and Stafford [1930] and McMichael [1932].

The investigations of Cameron and Mayes lend support to the view that the greater part of the blood from the hepatic arterial supply first traverses a capillary network in Glisson's capsule, from which it is collected by internal hepatic radicular venules which then join the portal stream: direct arterio-venous anastomosis is excluded by them, as also by Olds and Stafford, though the latter postulate a considerable direct communication of arterial capillaries with the portal sinusoids at the periphery of the lobules. According to their view, there are at the extreme periphery of the lobules some sinusoids containing only portal blood, and some only hepatic artery blood: these, however, intercommunicate further on, so that, at about 3–7 cell diameters from the periphery of the lobule, the two streams mix freely. It appears to us that our results are explicable on either of these views, and would indeed agree with the results of most other recent work which excludes the possibility of direct arterio-venous anastomoses. Our results, moreover, seem to point to the conclusion that, since the glycogenic power of the liver is not immediately impaired by reduction in its oxygen supply, the subsequent loss of glycogen which is evident about 12 hours after occlusion of the arterial supply may be secondary to changes in the circulation in Glisson's capsule. It is possible, however, that the deprivation of oxygen may not really be so great as would at first appear from the statements regarding the participation of the hepatic artery in the total blood flow through the liver. It was shown by McMichael that clamping of the hepatic artery only caused a slight fall in portal pressure, and Bauer *et al.* showed that it was accompanied by an increased inflow by the portal vein and that there was a measure of reciprocity between the two flows, so that the outflow, when both supplies were available, was less than that calculated from the sum of the two isolated supplies. Under the conditions of our experiments, moreover, this reciprocal effect would be likely to be enhanced, since occlusion of the hepatic and gastro-duodenal arteries would probably cause blood to be directed to those abdominal organs

which drain into the portal veins. Lastly, there might still be some arterial supply from the collateral circulation with diaphragmatic arteries, though this would probably be a small one.

(b) Reduction of the portal inflow.

The whole blood flow through the liver is certainly large, and is estimated by Grab, Janssen and Rein [1929] at from 50 to 60 p.c. of the total return by the inferior vena cava. Even if we accept a high figure for the hepatic artery inflow, we are left with a portal inflow of the order of 40 p.c. of the inferior cava current. As it was not proper, for obvious reasons, to attempt to reduce the portal inflow by direct constriction of the portal vein, we effected it by tying off various arteries of supply to the splanchnic area, or by partial evisceration.

In the experiments of arterial ligation we tied off either the superior mesenteric artery or the cœliac axis, or both, causing as little damage as possible to the periarterial nerve plexuses. When either vessel was tied off, there was only an evanescent duskiness of the intestines, since anastomotic connections are very free. When the superior mesenteric artery was tied off, the liver received its arterial supply by the normal channels, as well as the portal return from the cœliac axis and inferior mesenteric arteries. This procedure was shown by McMichael to produce a sudden and considerable fall in portal pressure, much greater than that produced by clamping the hepatic arteries, though it is uncertain to what extent the reciprocal adjustment of circulations, referred to above, may compensate for the reduced portal inflow by a greater hepatic artery supply. However, the fact remains that in two experiments of this type, both showed a remarkably good return of liver glycogen, at least for a time. One of these experiments is shown in Fig. 4.

The cœliac axis was tied close to the aorta in one experiment. In this the liver still received an indirect arterial supply from the superior mesenteric artery by way of the anastomotic gastro-duodenal artery (*vide* Fig. 2) as well as the normal or even enhanced portal return from the territory fed by the superior mesenteric artery and the small inferior mesenteric tributary. In this experiment the return of glycogen was also very prompt and large, though it fell away later as was the rule in many control experiments. This experiment is shown in Fig. 5.

When the cœliac axis and the superior mesenteric arteries were both tied, leaving only the insignificant portal return from the inferior mesenteric artery to supply the liver, the liver glycogen, as might be anticipated, fell off rapidly and showed no return (Fig. 6). As the blood sugar also

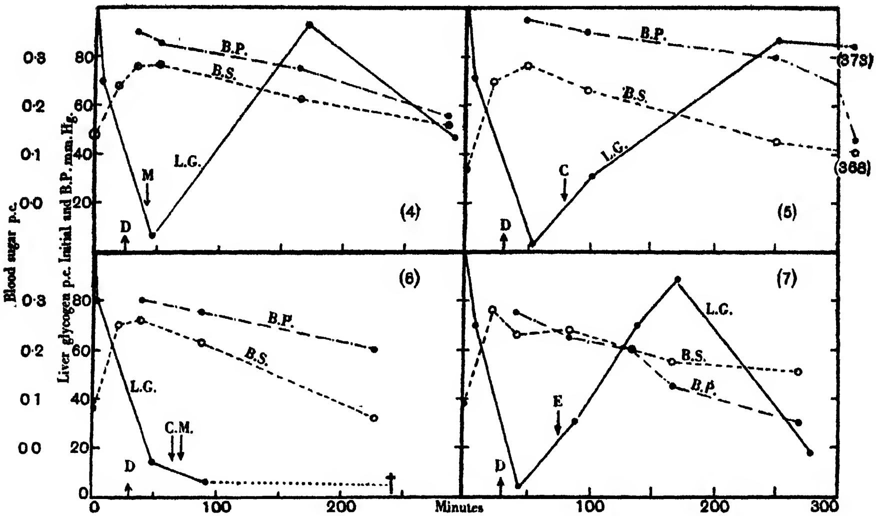

Fig. 4. Cat. 2·45 kg. Fasted 44 hours. Decapitation at 24 min. followed by ligation of superior mesenteric artery (M) at 41 min. Calculated initial liver glycogen = 2·12 p.c. Percentages at other times (ether at 0) were:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
5	1·59	171	1·98
45	0·14	290	1·00

Fig. 5. Cat. 3·0 kg. Fasted 48 hours. Decapitation at 30 min. followed by ligation of cœliac axis (C) at 78 min. Calculated initial liver glycogen = 2·13 p.c. Ether at 0 min. Glycogen concentrations at other times:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
6	1·53	250	1·84
53	0·06	373	1·60
101	0·66		

Fig. 6. Cat. 3·4 kg. Fasted 44 hours. Decapitated at 30 min. Ligation of cœliac axis and superior mesenteric artery at 64–71 min. Calculated initial liver glycogen = 1·48 p.c. Glycogen concentrations at other times:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
3	1·18	92	0·09
49	0·21	234	(?)0·00

The animal died between 234th and 292nd min.

Fig. 7. Cat. 3·3 kg. Fasted 44 hours. Decapitated at 29 min. Partial evisceration (see text), completed (E) at 75 min. Calculated initial liver glycogen = 1·58 p.c. Glycogen at other times:

Min.	Glycogen p.c.	Min.	Glycogen p.c.
8	1·11	139	1·11
43	0·06	171	1·41
89	0·48	269 and 279	0·22 and 0·31

fell quite rapidly, it was probable that we were dealing, in fact, with a virtually completely eviscerated preparation.

In the partial evisceration experiment we removed the spleen, and the intestine with its mesentery from the third part of the duodenum to the rectum, and ligated the superior mesenteric artery. There thus remained the normal (or enhanced) hepatic arterial supply and the venous return from stomach, pancreas and duodenum. Here again there was excellent recovery (Fig. 7).

On the whole these experiments with restriction of the portal flow show that there is considerable latitude in the vascular requirements of the liver. It is tempting, since the recoveries of glycogen were at first all rather above the average performance, to suppose that the restriction of venous return has, by allowing of a larger relative arterial supply, or in some other way, actually augmented the rate of recovery, However, such a conclusion is hardly justifiable without further experiments to support it.

In one experiment, after decapitation, we made a "reduced preparation" by tying off the aorta just above the origin of the inferior mesenteric artery, and the inferior vena cava just below the entrance of the renal veins. This was intended as the preliminary to an attempted visceral preparation similar to that described by Markowitz and Essex [1930], which consists of heart, lungs, alimentary canal, pancreas and liver. The preparation gave fairly good recovery up to 155 min., but the arterial pressure then rapidly fell and the liver glycogen was quickly lost again.

We also got fair recovery in another experiment in which we effected still more reduction by tying off both axillary arteries at their first part, and the aorta just below the superior mesenteric, the corresponding veins not being ligated: in this instance the arterial pressure and liver glycogen were well sustained until about 170 min. These experiments were not further pursued, however, because, although it was evident that a transfusion of some kind was desirable in order to maintain proper circulatory conditions, we had not at that time much knowledge of the effect of defibrinated blood, or of anti-coagulant materials, on the recovery process. These points will be discussed later.

(2) Effect of occlusion of the bile ducts.

It was shown by Bernhard [1931] that when the common bile duct is ligated in rats the animals survive longer when given glucose injections. This he attributes to deposition of liver glycogen. We carried out three experiments in which, after decapitation, the common bile duct and cystic duct were tied off (Fig. 2 *c* and *d*). All gave similar results. By the

end of the experiments (about 7 hours in two cases; death at 4 hours in one) there was considerable distension of the hepatic ducts. The following is a protocol of the most typical experiment:

Exp. 201. Cat ♂. 4·3 kg. Fasted 48 hours.

Time min.	Procedure	Arterial pressure mm. Hg	Blood sugar mg./100 c.c.	Liver glycogen actual p.c. (mean of 2)	Liver glycogen p.c. of initial
−2	Ear puncture	—	95	5·16 (calc.)	100
0	Ether given	—	—	—	100
5		—	—	3·88	75
9	Respiration stopped. Gave A.R.	—	—	—	—
13		—	273	—	—
18	Decapitation completed. A.R. by Schuster pump, 750 c.c. at 19 strokes, per min.	—	—	—	—
25		120	302	—	—
42	Common and cystic ducts ligatured	—	—	—	—
44		—	—	0·71	14
47		—	—	0·72	14
52		125	261	—	—
186		95	269	—	—
190		—	—	2·23	43
286		85	184	—	—
293	37 c.c. urine drawn (trace of bile?)				
295		—	—	3·08	59
376		55	143	—	—
426	(4 liver samples)	—	—	2·05	40
437		35	118	—	—
440	Killed. P.M.				

Wt. of liver left = 59 g.
Initial wt. of liver = 79 g.

The experiments all showed that for some hours after ligation of the bile ducts recovery of liver glycogen is still possible. The recovery in the experiment given above is perhaps rather on the low side, even when the rather high initial glycogen content is considered. In another experiment with the low initial glycogen concentration of 0·38 p.c. the recovery was, however, well above the average and reached 190 p.c. of the initial value in 168 min., though this was not sustained. On the whole, we may say that for the first 2 or 3 hours occlusion of the bile ducts makes little difference to glycogen storage in the liver of the decapitate cat.

III. Effect of alterations in the amount or composition of the circulatory blood.

It was found by Evans, Tsai and Young [1931 *b*] that, when compared with the rapid rate of spontaneous recovery of glycogen after decapitation, the effect of infusion or other exhibition of glucose had a

relatively slight effect on the glycogen formation. This was surprising, because the readiest assumption was that the glycogen which accumulated so rapidly in the liver after decapitation was formed principally from the blood sugar, which was always high just after decapitation, and which, in fact, did fall during recovery. They not unnaturally expected that if this were the case, the addition to the blood stream of a considerable amount of glucose would not only greatly accelerate the speed at which glycogen was laid down, but would lead to the attainment of concentrations of liver glycogen considerably beyond those present to begin with. Since neither of these things happened it was concluded that either the circulating precursor was not, or not wholly, blood and tissue glucose, or else that the glycogen was formed from cleavage products which had been stored, not in the blood and tissue fluids, but in the liver itself, or in some other tissue. The possibility that the precursor of glycogen was present in the blood in the form of a hexose phosphoric ester, or as glucose *plus* a phosphate, was investigated by De Graff, Evans and Vacek [1932] and rejected. We have, rather as a forlorn hope, tried the infusion of an amino-acid and of products of starch breakdown, with saline infusions as controls.

(1) *Infusion of saline solutions.*

Six experiments were carried out in order to provide controls for the other experiments on infusion. In these the initial (calculated) glycogen concentrations ranged from 1·61 to 3·54 p.c. (mean 2·52 p.c.), so that the results, expressed in percentage change, should be comparable with those of the average curve for a large number of fasted cats. After decapitation, 25 c.c. of warm 0·9 p.c. NaCl solution in tap water was run into the femoral vein, from 10 to 20 min. being taken for the infusion. The results were not very uniform; in three cases the recovery rate was quite definitely greater than the average, in two it was about the same for 140 min. and then fell off, and in one it was poor throughout. The mean curve for the six experiments is given below (Fig. 8). It shows that, even when the average is taken, there is a definitely greater rate of recovery than in controls with no infusion and with about the same average initial glycogen content. The blood sugar also fell a little more quickly, but there was no very evident diuresis.

If these results can be interpreted to mean that the infusion has brought about an acceleration of glycogen recovery, as we think they can, it is probably better for the present to accept the fact and to let the explanation wait. Several possible ones suggest themselves, but have not been examined.

(2) *Infusion of alanine.*

The formation of glycogen from protein has long been accepted, and it has recently been shown by Wilson and Lewis [1930] that *d* or *d*-*l*-alanine, when given by the mouth to fasting rats, led to an increase of liver glycogen in 3 hours. It seemed worth while exploring the possibility

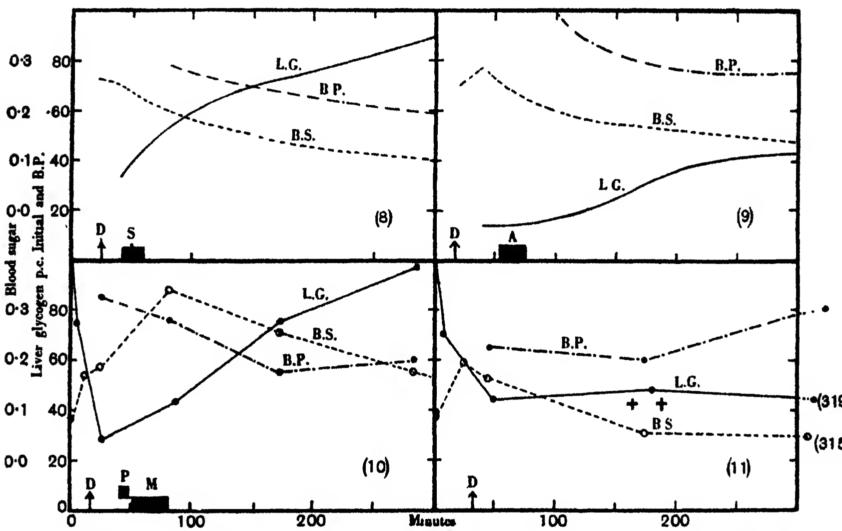

Fig. 8. Mean curve for six experiments in which 25 c.c. of saline solution were infused at *S*. *L.G.* liver glycogen; *B.S.* blood sugar; *B.P.* arterial pressure; *D*, decapitation.

Fig. 9. Mean curve for three experiments. Decapitate cats, infusion of alanine (2 g., 2 g., 4 g.) in 25 c.c. saline into femoral vein at *A*.

Fig. 10. Infusion of 2·5 g. of dextrin-maltose mixture at *M* into portal circulation of decapitate cat. Initial glycogen concentration = 1·68 p.c. *D*, decapitation; *P*, removal of spleen and insertion of cannula into splenic vein.

Fig. 11. 2·4 units insulin 21 min. before ether given. *D*, decapitation. Initial glycogen 5·23 p.c. Blood sugar before insulin = 0·083 p.c., 19 min. after = 0·085 p.c. The crosses show where series of convulsions occurred.

that, in our animals, glycogen might rapidly be producible from amino-acids, and, if this proved to be the case, it was conceivable that the glycogen formed in recovery might also arise from protein or from amino-acids.

Out of six experiments, a solution of *d*-*l*-alanine (B.D.H.) (2–4 g. in 25 c.c.) was infused, into the portal vein in one decapitate cat, and into the femoral vein in four decapitate and one decerebrate cat. The kidneys were first removed from one of the decapitate cats, and the urine of the three others was examined by formalin titration for alanine; in these

0·13 g. out of 2·5 g. injected, 0·38 g. out of 4 g. and 0·7 g. out of 3 g. were respectively found in the urine, and as these estimations included ammonia with the amino-acids, we may take it that less than 20 p.c. of the infused alanine was excreted. There was no evident dehydration of the tissues in these experiments.

The results of the experiments showed a slow loss of liver glycogen in the case of the decerebrate cat; in the five decapitate animals there was an increase which varied between wide limits. When all five experiments were averaged the curve was approximately the same as that given for untouched control experiments by Evans, Tsai and Young. But this result is largely due to the fact that one of the experiments gave an increase to 214 p.c. of the calculated initial value; this result should probably be rejected, for reasons already explained, on account of the low initial liver glycogen (0·42 p.c.). Another experiment (the portal vein infusion of 2 g. alanine) showed a slight loss of liver glycogen, but as this had an initial content of 7·85 p.c. glycogen, it might also be excluded. The remaining three, with initial glycogen from 2·5 to 3·5 p.c., which might be considered comparable with the saline injections, gave recoveries definitely below the average for non-injected animals and much lower than those injected with saline solutions, as is seen from Fig. 9. The blood sugar curve also fell rather less quickly than usual. The well-sustained arterial pressure shows that the loss of glycogen was not due to a poor circulation.

These experiments certainly do not lend any support to the idea that glycogen can rapidly be formed from alanine in the decapitate cat when it is injected into the general circulation, and we have not pursued them further.

(3) *Infusion of products of digestion of starch.*

In two experiments an attempt was made to find whether glycogen could be rapidly built from some of the intermediate carbohydrates, since it is possible that some of the products of glycogenolysis which may be present in the blood after decapitation might consist of larger molecules than glucose. The experiment had further interest in the possibility that such larger molecules might form part of the normal absorption products from the digestion of starch, suggestions of the presence of which in the portal blood have been made by several of the earlier observers (*e.g.* Pavy).

For the infusion, a mixture of maltose and dextrins was prepared by acting on soluble starch solution with saliva at body temperature until

the achromic point was reached. The solution was then boiled, filtered and diluted down to make a 10 p.c. solution relative to the initial starch. The results of one experiment, in which 25 c.c. of solution were slowly infused into the portal (splenic) vein, are given in Fig. 10.

The results are similar to those reported by Evans, Tsai and Young for the injections of glucose into the portal vein and do not differ significantly from those in which a simple saline solution was infused.

It seems doubtful, therefore, whether, in the short duration of these experiments, the natural rate of restitution can be accelerated by either glucose or malto-dextrins. In the later stages, not shown in the graph, the glycogen content of the liver, as usual, declined again.

(4) *Previous administration of insulin.*

In view of the possibility that insulin may, under certain conditions, retard glycogenolysis in the liver, it was thought that there might be an increased amount of insulin present after decapitation, and that this might favour the deposition of liver glycogen, and, though both parts of this supposition seemed unlikely, we put it to the test. In a preliminary experiment we found that the subcutaneous administration of 2·4 units of insulin to an unanæsthetized fasted cat produced a slow lowering of blood sugar, from 0·076 p.c. initial to 0·063 p.c. in 1 hour and to 0·04 p.c. in 5 hours, and we accordingly chose this dose as being not too great for an acute experiment under our conditions. We did three such experiments, giving the insulin 20 min. before the etherization for the subsequent procedure.

The results were as we anticipated (Fig. 11), viz. a smaller rise of blood sugar on decapitation, a subsequent fall to a low level, and a retarded and small glycogen recovery (except in one case where the initial glycogen was very low). The loss on decapitation in both of the satisfactory experiments was not very great, which favours the suggestion that the insulin had checked the glycogenolytic process.

Though we might have tried the effect of smaller amounts of insulin, we did not think this worth while at the present juncture.

(5) *Replacement of the circulating blood by defibrinated blood.*

These experiments were undertaken because of their relation to perfusion experiments, for which defibrinated blood is so generally used. Evans, Tsai and Young [1931 *b*] found that glycogen recovery was favoured by infusions of defibrinated blood when this resulted in an improvement of a poor circulation. We have carried this further by

giving defibrinated blood, not only to supplement that already in circulation (four experiments), but also (in two experiments) to replace it as completely as possible, by several successive infusions and bleedings. The defibrinated blood was obtained from a second cat which was bled out in two or three stages for the purpose, the liver glycogen being followed to ascertain the effect of severe hæmorrhage upon it. The defibrinated blood when freshly drawn usually had a high sugar content, but this was not invariably so by the time the infusion was made, because of loss in the meantime by glycolysis.

It is not necessary to give details of the experiments in which defibrinated blood was added to that already circulating. It will suffice to say that they all showed recovery, though this was no greater than was to be expected without such additions. One of the effects of the infusion was a great enlargement of the spleen, and it would probably have been better to have removed this organ beforehand in such experiments.

The following examples will suffice to show that even after the substitution of the larger part of the animal's own blood by defibrinated blood, good recovery can still occur. This is rather remarkable in view of the severity of such treatment. The protocol for a donor animal (from a different experiment) also illustrates the rapid loss of glycogen during bleeding.

The effect of hæmorrhage on the glycogen content of the donor is of interest since it explains why such low initial glycogens have been obtained by those who have attempted to prepare the liver for perfusion, or for analysis, by methods involving considerable loss of blood. There can be no doubt that the loss is principally due to attendant liberation of adrenaline and to nervous influences from the anæmic centres acting directly upon the liver.

Exp. 205. *The donor.* Cat. 2·15 kg. Fasted 44 hours.

Min.		Blood sugar p.c.	Liver glycogen p.c.	Liver glycogen p.c. of initial
−1	Blood by ear puncture	0·115	3·2 (calc.)	100
0	Ether given	—	—	—
6		—	2·31	72
16		0·336	—	—
17–19	52 c.c. bled from carotid	—	—	—
20		—	0·61	19
28–31	Further bleeding, 10 c.c.	—	—	—
32		—	0·23	7
34		—	0·16	5
36	Killed			
99	Analysis of defibrinated blood (just before infusion)	0·329		

Exp. 212. *The recipient.* Cat. 2·45 kg. Fasted 44 hours.

Min.		Arterial B.P. mm. Hg	Blood sugar mg./100 g.	Liver glycogen p.c.	Liver glycogen p.c. of initial
−1	Blood by ear puncture	—	92	1·43 (calc.)	100
0	Ether given	—	—	—	—
9		—	—	0·93	65
14		—	253	—	—
25	Decapitation completed	—	—	—	—
31		135	275	—	—
35		—	—	0·45	31
48	Infusion of blood (0·351 p.c. glucose) into femoral vein begun	—	—	—	—
54	23 c.c. now run in, continued	160	—	—	—
62	Bleeding begun, infusion continued	—	—	—	—
71	Bleeding stopped (45 c.c. in all)	—	—	—	—
72	Infusion stopped (65 c.c. given)	75	—	—	—
74	Infusion of cats own defibrinated blood (0·324 p.c. glucose)	—	—	—	—
88	Infusion stopped (57 c.c. given)	145	—	—	—
93	Bleeding begun	—	—	—	—
95	Bleeding stopped (40 c.c.)	135	—	—	—
98	Infusion begun	—	—	—	—
103	Infusion stopped (32 c.c.)	—	—	—	—
113		—	—	0·90	63
116		115	299	—	—
238		65	115	—	—
243		—	—	3·14	219
331		—	—	2·34	166
336		60	101	—	—
400		52	080	—	—
405		—	—	2·67	186
437		40	089	—	—
439		—	—	0·57	40
441	Killed. Liver remaining = 54 g.				

In the infused animal the circulating blood was almost wholly replaced by defibrinated blood, as was shown by its incoagulability when drawn; but towards the close of the experiment slight coagulability had returned to it. In the other experiment there was less recovery than in this case, apparently because the initial glycogen was higher (5·75 p.c.), but all these experiments may be taken together as showing that the defibrinated blood exerts no peculiarly deleterious action on the glycogenic power of the liver. The chief drawback to defibrinated blood is the vascular shock which it produces. This is shown in Exp. 212, given in full above, by the fact that although the blood-pressure at 95 min. was 135 mm. Hg, and as a further 32 c.c. were subsequently infused giving the

animal finally 69 c.c. more blood than at the start, the pressure by 116 min. had fallen to 115 mm. Hg, and in subsequent periods was not well maintained. It was for this reason that we proceeded next to try the effect of various anti-coagulant substances on the glycogen restoration.

(6) *Addition of heparine.*

Infusions of heparine [Messrs Hynson, Westcott and Dunning], dissolved in 25 c.c. normal saline solution, were given into the femoral vein in three experiments. The doses used were 7·4, 14·8 and 29·4 mg. per kg. body weight. The blood in the first experiment clotted *in vitro* in 10 min., that in the other two remained incoagulable for 24 hours.

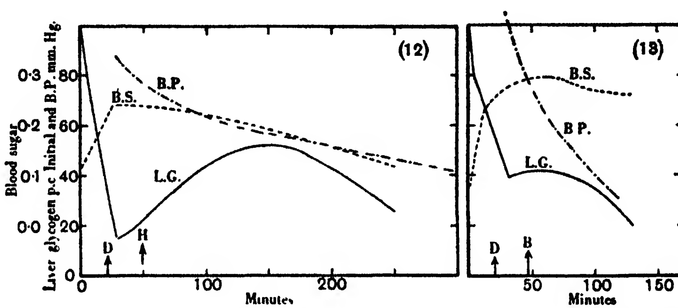

Fig. 12. Mean curve of three experiments in which heparine was infused.

Fig. 13. Mean curve of two experiments in which 6 c.c. of 8 p.c. Chicago blue were injected intravenously at B. Decapitation at D.

Recovery of glycogen was below the normal for the two higher doses and above for the smallest dose, but the initial liver glycogen was low in that case, so that supernormal recovery was to be expected. When averaged out the three curves gave recoveries below the normal average (Fig. 12). It would seem that the use of heparine is detrimental to the liver.

(7) *Addition of Chicago blue.*

Two cats, subsequent to decapitation, were infused with 6 c.c. of 8 p.c. Chicago blue [Rous, Gilding and Smith, 1930], previously boiled and filtered, and washed into the femoral vein with a small quantity of saline. There was loss instead of recovery of liver glycogen, the hyperglycæmia showed little reduction, the blood-pressure fell rapidly and the animal soon died (Fig. 13). The sample used appeared to be toxic to the liver, and further investigation was abandoned owing to the drawback this anticoagulant has of preventing visual assessment of the degree of arterialization of the circulating blood.

Summary.

1. Further consideration is given to the details of the methods used for the taking of liver samples for glycogen estimation. The changes observed after decapitation are not attributable to changes in the water or fat content of the liver. In experiments involving the taking of several successive samples of liver it is important, where changes of the total glycogen content of the liver are required ("balance-sheets"), to take into consideration the effect of the diminishing amount of liver tissue present, which makes glycogen gains smaller than they appear to be.

2. Livers of fasted cats with low initial glycogen content show on recovery from decapitation a greater percentage gain, relative to the initial content, than do livers of similarly fasted cats with high contents to begin with. Hence it is only when the initial glycogen concentrations are comparable that it is possible, in different experiments, satisfactorily to compare the liver glycogen changes, expressed in terms of initial glycogen content.

3. Ligation of the hepatic artery does not inhibit the glycogen deposition after decapitation; considerable reduction of the portal inflow, by ligation of the cœliac axis or superior mesenteric artery, or by partial evisceration (removal of spleen and intestine) also does not interfere with the recovery, but simultaneous ligation of cœliac axis and superior mesenteric artery does stop recovery. Occlusion of bile exit along the bile ducts also does not inhibit recovery.

4. Infusion of 25 c.c. of normal saline solution into the femoral vein somewhat enhanced recovery, but infusion of 2–4 g. of alanine delayed it. Recovery of glycogen was not greatly, probably not significantly, increased by injection of 2·5 g. of a malto-dextrin mixture into the portal vein. Subcutaneous injection of 2·4 units of insulin 20 min. before the experiment was begun retarded recovery.

5. Replacement of the circulating blood by defibrinated cat's blood did not inhibit the recovery significantly, but injection of heparine or Chicago blue in anti-coagulant doses did inhibit it, the former slightly, the latter, with the sample at our disposal, completely.

We wish to thank Dr H. P. Gilding for the sample of purified Chicago blue.

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THE EFFECT OF SOME ACCIDENTAL LESIONS ON THE SIZE OF THE SPLEEN.

By JOSEPH BARCROFT.

(*From the Physiological Laboratory, Cambridge.*)

Observations made by Barcroft and Florey [1929] showed that the operation for exteriorization of a portion of the small intestine caused a shrinkage of the spleen which persisted for days and perhaps weeks after the actual operation. Moreover, the shrinkage was accentuated by perforation of the gut leading to peritonitis.

Further observations were made in which the abdomen was opened [Barcroft, 1931], a loop of intestine was exposed for a few minutes under aseptic precautions and replaced in the abdominal cavity, which was then closed. The result was a contraction of the spleen lasting for some days.

These operations may be contrasted with such a lesion as a hernia, which is followed by no contraction of the spleen. Fig. 1 shows the alteration which took place in the volume of the organ, (1) when the operation of excision of the superior cervical ganglion was performed, (2) when a hernia developed just ventral to the spleen, so that the abdomen was closed only by the integument, and (3) when the operation was performed successfully to cure the hernia. In the cases (1 and 3) of operation there was a marked contraction of the spleen which lasted some days. In the case of the hernia, however, caused presumably by a stitch giving way in the region of the spleen, there was no contraction. We do not know the precise stimulus which was responsible for the contraction. In the present case it was presumably a chemical one, for the spleen had been denervated. But granting the chemical nature of the stimulus, there remains unsettled the question of whether the stimulant was a product of tissue breakdown, of bacterial action, or was adrenaline which might have been liberated as the result of some action of nervous origin on the supra-renal bodies.

Four experiments were performed [Barcroft, 1931] in which the superior cervical ganglion was excised. In two cases the spleen had been

denervated during exteriorization, and in two others it had not. In all cases the spleen contracted as the result of the operation on the neck. The contraction was more marked in the innervated spleen, but not significantly so. It was hoped that by observation of the pupil on the side from which the ganglion had been removed, some information would be obtained as to the presence in undue amount or absence of adrenaline

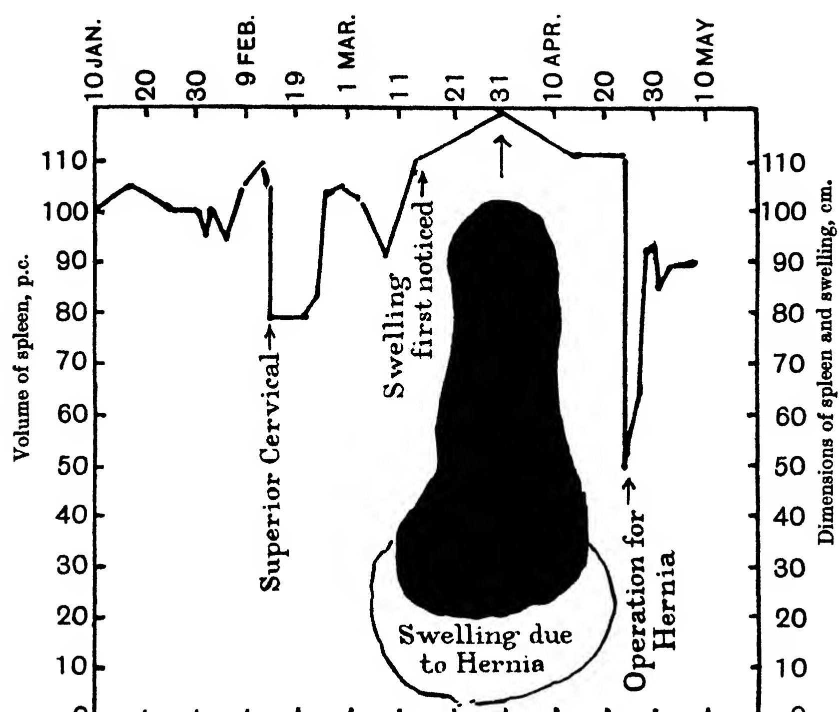

Fig. 1. Volume of spleen following (1) excision of superior cervical ganglion, (2) development of a hernia, (3) operation for hernia. Spleen reduced to seven-twelfths of its linear dimensions.

in the blood. This hope was not fulfilled, the dog being an unfavourable animal for observations on the sensitization of the pupil to adrenaline.

Another dog (Nina), the spleen of which was denervated during exteriorization, also yielded an interesting result. The superior cervical ganglion was removed and the spleen contracted and was regaining its normal size when, nineteen days after the operation, it again suddenly shrank; the bandage had caused a considerable abrasion under the neck.

It took nine days for the wound to heal, during which time the spleen remained shrunken and relatively colourless. Apart from the fact that the spleen had been denervated, the interesting points about the lesion were that it was purely superficial and not severe (Fig. 2).

During the winter of 1931–2 a phenomenon appeared in a number of animals which suffered from necrosis of considerable areas of skin. The necrosis developed only in those animals on which operations had recently

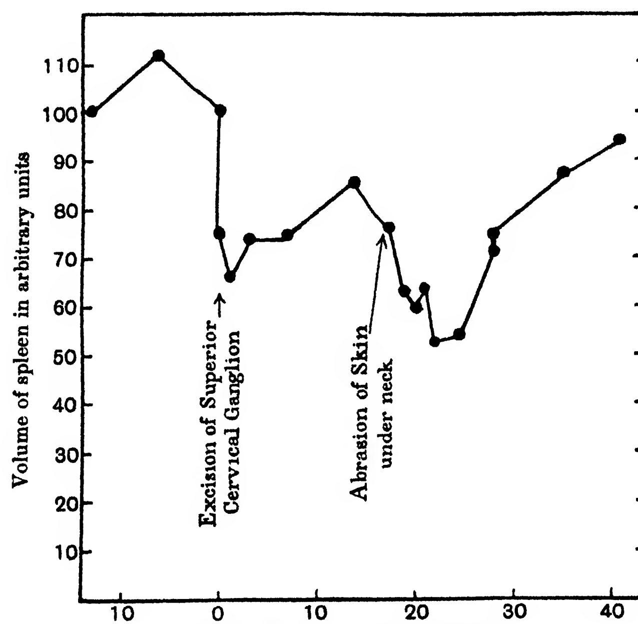

Fig. 2. Changes in volume of spleen following (1) excision of superior cervical ganglion, (2) abrasion of skin under neck.

been performed. Usually about a week, but in one case three weeks, elapsed before it became evident that a large patch of skin was dead and had to be removed. When that was done and the bare area dressed systematically the animals made a healthy recovery. The injury was quite superficial and the animals did not appear to be in pain, their spirits and appetites being excellent.

Without going into the various possibilities which might account for this phenomenon, the fact that it was confined to a certain category of animals ruled out a general epidemic. Experiments were instituted to test whether the operation had so lowered the resistance of the animals

that they fell a prey to infection which other dogs defied. The first of these experiments showed the true nature of the trouble. In order to obtain controls two animals were given an anæsthetic each for an hour and subjected to all the routine procedure of an operation, but no operation was actually performed upon them. These two, so far from experiencing no trouble, were much more seriously affected than the rest. The areas of necrosed skin were larger, the damage went deeper and appeared earlier. The operating table was of the type in which a copper top is warmed by an electric bulb beneath. Although the animal lay on perforated zinc normally separated by an air space from the copper and with a sterilized cloth between its body and the zinc, the events showed that the surface of the skin had been exposed to too high a temperature. Clearly this accident could not have occurred with the type of table kept warm by means of a hot-water tank, as the effect depends upon the production of heat at a rate faster than it is conducted away.

The most severe case, that of Nina, may be taken first.

Protocol. Jan. 20, 1932. Nina (spleen exteriorized and denervated Nov. 20, 1929) was placed on the dissecting table under C.E. mixture, as when animals are normally operated on for exteriorization of the spleen. The perforated tray and cloths were between her hair and the table. No operation was performed. She lay so for an hour. The spleen shrank in the usual way under anæsthetic.

Jan. 22. Nina was somewhat stiff in her gait and dull in general appearance. The hair on the right side was darkish looking as though a burn might develop, but as yet there was nothing further to be seen.

Jan. 23. Temp. 103·8° F. Skin nowhere broken, but two areas obviously necrosed. They were congested and the hair was loose. One area was in the flank, the other between the ribs and the thigh.

Jan. 24. Temp. 102·2° F. Affected areas as in Fig. 3.

Jan. 25. The care of the areas was handed over to Major Linton, R.A.M.C., who did the subsequent dressings, removing the necrosed skin and tending the affected areas with Eusol.

Jan. 26, 27 and 28. The bare areas extended and it became evident on the 28th that the damage had penetrated to the muscle. Temperature rose from 102° to 103·6° F.

Jan. 29. Temp. 88·5° F. No appearance of pain. Vomited whilst being dressed; no appetite.

Jan. 29–30. Dog died.

It is worth putting on record, though of no great interest in the present connection, that the mental condition of the animal, though lacking its usual brightness, was quite tolerable, and apparently equal to that of many rather phlegmatic dogs. On both January 28 and 29 she took interest in what was going on about her, her rectal temperature on the 28th being 103·6° and on the 29th 88·5° F. On the latter day her

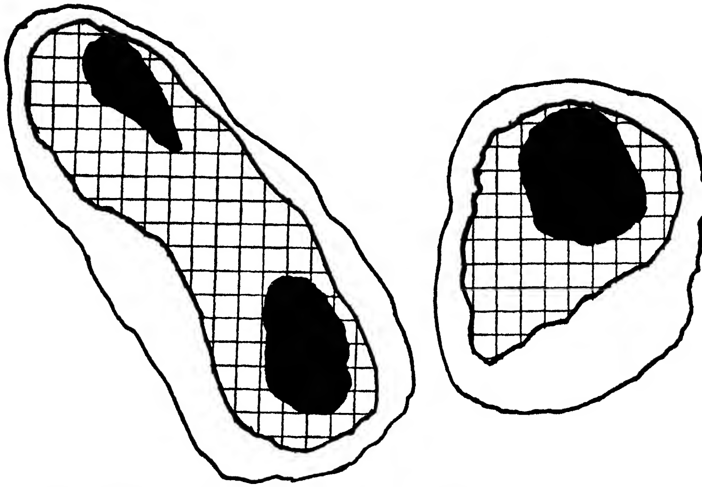

Fig. 3. Affected areas of skin in Nina. Black = skin dark. Cross-ruled = hair falling out (each square = 1 sq. cm.). Outer part = skin probably affected.

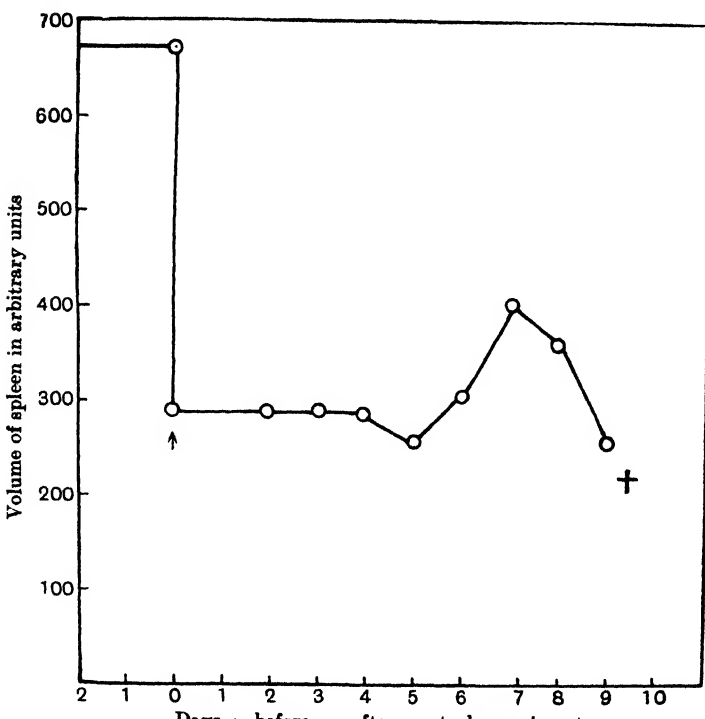

Fig. 4. Changes in volume of spleen of Nina after control experiment.

condition appeared bright enough to make me doubt the thermometer reading, and indeed not until the temperature had been taken three times and by two different thermometers did I accept it. Thus over a range of 15° F., or more than 8° C., the animal was quite *compos mentis.* The changes in volume of the spleen are shown in Fig. 4.

A similar experiment was performed on Bell on Jan. 21st, *i.e.* before the result of that on Nina had revealed itself. Bell differed from Nina

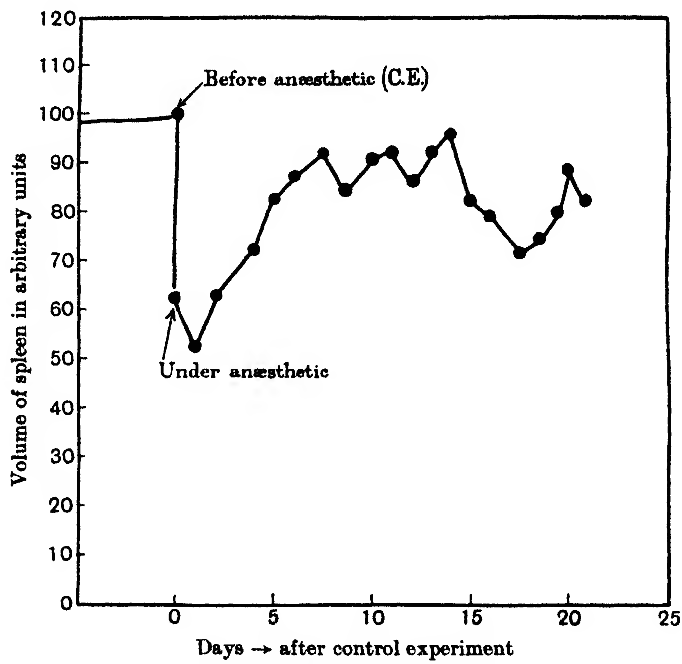

Fig. 5. Changes in volume of spleen of Bell after control experiment.

in three respects: (1) the spleen was not denervated, (2) the animal recovered, (3) the spleen only remained in a state of extreme contraction for a few days.

The lesions were of the same general nature as those of Nina, *i.e.* one in the flank and another behind the ribs.

The first portions of dead skin were removed on January 26th and the wound kept clean with Eusol. The animal "ran" rather a high temperature.

Jan. 27	Jan. 28	Jan. 29	Jan. 31
105° F.	104·2° F.	105° F.	103° F.

Protocol. Procedure as with Nina and earlier observations similar.

Jan. 31, 1932. Both areas were reported clean and the surfaces healthy and granulating.

Feb. 15. Area (1) flank dressed with amyl salicylate, area (2) with vaseline. This was continued up to Feb. 22, when the amyl salicylate dressing was stopped and both wounds were dressed with vaseline. In judging of the amyl salicylate dressing it should be said that while no benefit was derived from it, two facts must be borne in mind, firstly it was not used from the start and secondly it seemed less soothing—the dog was inclined to rub the area dressed with salicylate and not that dressed with vaseline.

Feb. 29. Area (1) healed, (2) healing rapidly.

Mar. 3. Both areas healed.

The measurements of the spleen, Fig. 5, show that the maximum of shrinkage was on the second day after the injury, that is to say, before the skin had broken, and that at the end of a week the spleen had returned almost to its ordinary size, even though there were two large granulating surfaces.

In the light of these two experiments it is now possible to interpret some others in which the necrosed areas were much less extensive, and what is perhaps of interest, the necrosis was not evident for a much longer time after the exposure of the animal to warmth.

Protocol. Tilley II, spleen denervated and exteriorized on Dec. 17, 1931. The shrinkage of the spleen ran its usual course till the ninth day; the organ shrank rapidly till the fifteenth day, remaining contracted. On the twenty-first day the necrosis made itself evident and the dead skin was removed, the necrosed area cleaned thoroughly and dressed. By the twenty-fifth or twenty-sixth day the spleen was probably as large as it would have been had no skin lesion taken place.

Conclusions.

1. Necroses of the skin, caused either by friction or unduly high temperature, are accompanied by a contraction of the spleen.
2. The contraction may come on before the skin is broken and therefore before bacterial action from without has occurred.
3. The contraction may pass off before the necrosed area has "dried up" if it is suitably dressed.
4. The above effects may be observed in denervated as well as innervated spleens.

The thanks of the author are due to the Royal Society for a grant from which the expenses of the above research have been paid.

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612.413:612.63

ALTERATIONS IN THE SIZE OF THE DENERVATED SPLEEN RELATED TO PREGNANCY.

By JOSEPH BARCROFT.

(*From the Physiological Laboratory, Cambridge.*)

In a former paper [Barcroft and Stevens, 1928 *a*, *b*] it was shown that the spleen in dogs contracts during "heat" and again towards the end of pregnancy, the usual size of this organ at these periods being little larger than that which is normally observed during exercise. The question of whether this contraction was nervous or humoral, or both, was not discussed. Another point which was unsettled in that paper was the rate of recovery; in some cases it was almost immediate, in others it was less rapid.

The present paper deals with these points.

Operative.

Bitch Nina, weight 6·8 kg. The spleen was denervated during the operation for exteriorization on December 20, 1929.

First observed pregnancy. She came on heat on June 1, 1930, and took the dog on June 3, 4 and 5. Her pregnancy ran a normal course, with no mishap other than an abrasion of the neck caused by her collar on September 1. She had five living pups in the night September 3–4. Nina suckled these puppies; they died, however, one by one. On the 20th there were but two, on the 22nd one which she suckled till October 10 (Fig. 1 A).

Second observed pregnancy. The spleen remained fairly constant in size from October, 1930 to January, 1931, if anything there was a slight increase in size: on February 2 Nina showed signs of coming on heat. The effect on the spleen was rather ambiguous, *i.e.* there was a trifling contraction of the organ, but it was within the range of ordinary casual variations. In fact no change that could be connected with reproduction took place till the actual commencement of labour on April 23. On the morning of April 23 the area of the spleen was 29·5 sq. cm.; after labour commenced it was 26 sq. cm. and about ten minutes after the birth of the first pup it was 24 sq. cm. Six pups were born weighing in aggregate 1·3 kg. The area of the spleen was 23 sq. cm. next morning and it reached

its minimum of 21 sq. cm. on April 27. It varied between that and about 25 sq. cm. till May 16, when it tended gradually to return to its original size. On June 6 the pups were removed. For some days, however, they had supplemented such diet as they obtained from the breast by partaking of the food given for the mother (Fig. 1 B).

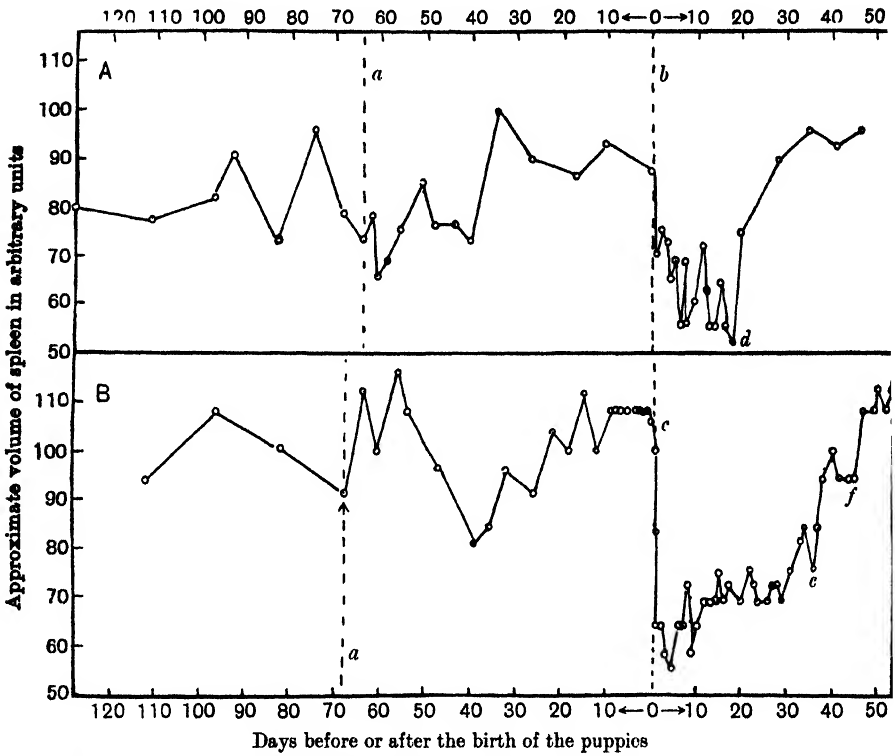

Fig. 1. Spleen denervated. A, first pregnancy; B, second pregnancy; (*a*) commencement of "heat," (*b*) five puppies born alive only one of which survived beyond the 18th day (*d*), (*c*) six puppies born alive of which four survived and were suckled entirely by the mother till the 36th day (*e*), and partially till 46th day (*f*).

Comparison of results obtained with innervated spleen.

The characteristic alterations in the size of the spleen, shown in Fig. 1, differ materially from those obtained in experiments on animals whose spleens had not been denervated. The outstanding facts are:

(1) In the denervated spleen there is no contraction during the later part of the pregnancy, whereas in the normal spleen the contraction in the last weeks is almost maximal.

(2) In the denervated spleen the alterations in size during "heat" are uncertain, whereas in the normal spleen there is well marked contraction.

(3) After parturition the spleen is contracted for a greater or less period whether it be denervated or not.

The present experiments seem to shed some light on the third of the observed phenomena. The contraction is evidently of uncertain duration, but it coincided pretty closely with the period of lactation. Further

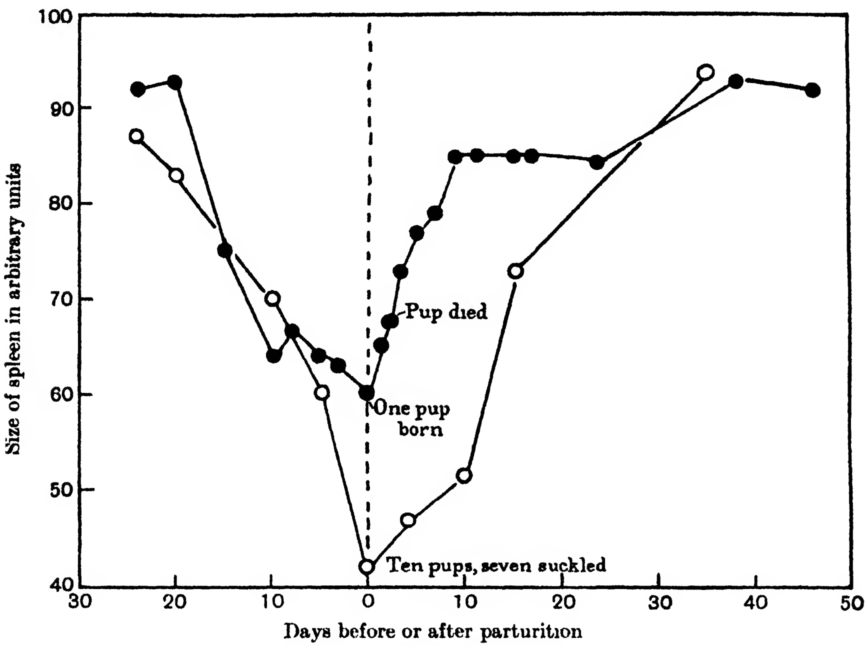

Fig. 2. Spleen innervated, correspondence between contraction of spleen and suckling of young.

evidence is to be found in support of this view from a consideration of some older experiments which were not understood at the time.

Fig. 2 shows the course of two pregnancies from about the sixth week onwards. In one the post-partural contraction was of short duration, in the other it was of long duration. In the first the bitch (Daisy) gave birth to but one pup which died on the third day, so that the demand for milk was practically non-existent; the other (Rastus) gave birth in the night to ten pups of which seven were found to be alive the next morning. She suckled the pups, but unfortunately no record was kept of the length of time.

Summary and conclusions.

1. The contractions of the spleen which are associated with pregnancy in the dog can be divided into:

(*a*) The "heat" contraction.
(*b*) The pregnancy contraction.
(*c*) The lactation contraction.

2. Of these the first two are abolished by denervation whilst the lactation contraction is undiminished. Presumably therefore there is a large humoral element in the causation of the lactation contraction whilst the "heat" and "pregnancy" contractions are nervous.

My thanks are due to the Royal Society for a grant which defrayed a portion of the expenses of this research.

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612.627.1:612.63

THE VOLUME OF BLOOD IN THE UTERUS DURING PREGNANCY.

By JOSEPH BARCROFT and PAUL ROTHSCHILD.

(*From the Physiological Laboratory, Cambridge.*)

THE discovery of Barcroft and Stevens [1928 *a*, *b*] that the spleen contracts during pregnancy, either to supply blood to the genital organs, or to contribute indirectly to the formation of blood in the fœtus or both, raises the question of the quantity of blood contained in the uterus during pregnancy. The present paper deals with that problem. Experiments were made on rabbits, cats and dogs. The present paper deals only with rabbits.

The experiments fall into two series according to the operative procedure employed.

Series 1.

The operative procedure was approximately the same for all. The animal was anæsthetized with C.E. mixture, 1 c.c. of blood was withdrawn from the carotid artery and made up to 50 c.c. The bladder was removed. A dissection was then made of the vessels which lead to the uterus. Usually there are five groups of vessels, one in the vagina, one on each side going to the broad ligament from the iliac vessels, and the ovarian vessels on each side going to the broad ligament from above. The general arrangement is given in Fig. 1[1].

A ligature was put round each of these groups of vessels loosely, with a "half-hitch." Great care was exercised lest the ligature should press upon the veins and cause congestion. At a given signal all the ligatures were tied simultaneously. If the operation was carried out successfully, all the avenues by which blood could enter or leave the uterus, vagina, Fallopian tubes and ovaries were closed simultaneously. A "Spencer-Wells" forceps was put on each group between the ligature and the animal, and the vessels were severed between ligature and forceps. The genital organs were then removed. No bleeding either from

[1] **Fig. 1 is purely diagrammatic, but in one respect it may be wrong. Since I commenced to observe this point I have never seen the same number of embryos on the two sides.**

the uterus or the animal should be caused by the removal. In case of bleeding from either the experiment is vitiated or, at least, there is a presumption of its being so. Should bleeding from the uterus occur, this would indicate an untied vein from which blood had already returned to the general circulation. In the case of bleeding from the animal there is the probability that an artery had been missed in tying the ligatures and that the uterus was therefore unduly congested. In the results

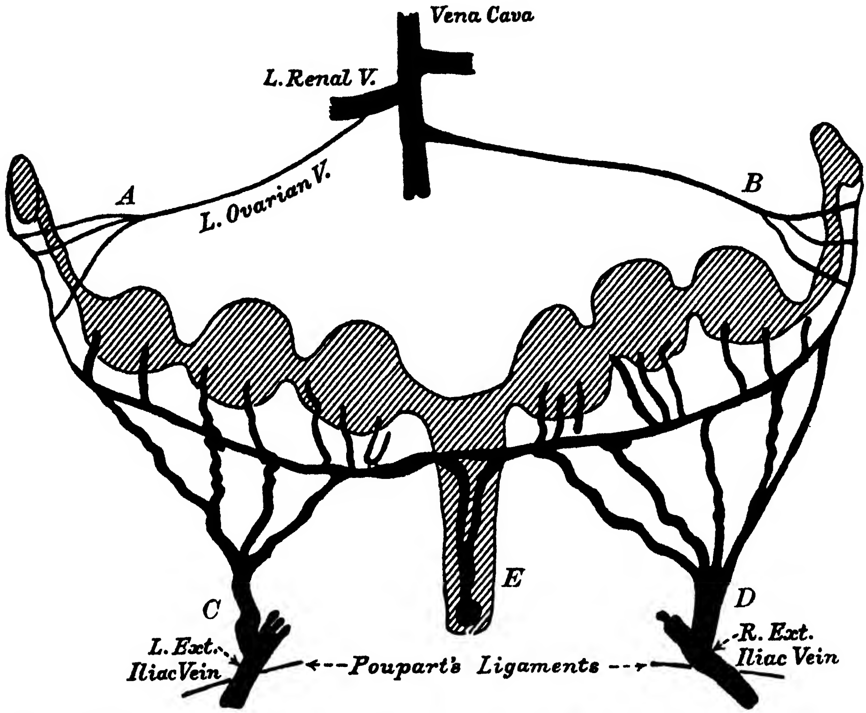

Fig. 1. Schematic representation of veins from female generative tract in pregnant rabbit.

appended no experiment has been included in which the genital organs did not come out cleanly. Four experiments have been omitted, on the ground that, since bleeding from the animal occurred when the vessels were cut, they all gave unduly high results.

The uterine vessels were opened up and washed out, then the uterus was cut up and thoroughly extracted with heparine saline. The extract, strained through muslin, was made up to a known volume and the blood present was estimated colorimetrically by comparison with the sample of blood taken at the beginning of the operation.

The results obtained from this series are tabulated in Table I and must be regarded as of a purely preliminary character, and indeed they might have been omitted entirely but for the facts (1) that they form an interesting basis of comparison with those obtained by the same technique on dogs and cats which will form the subject of a later paper, and (2) that certain individual experiments quoted in Table I will be referred to in the present paper.

TABLE I. Rabbits.

No. of exp.	Weight of animal kg.	Days since impregnation	Weight of uterus g.	No. of fœtuses	Wt. of fœtuses or young g.	Wt. of fœtal placenta g.	Blood in uterine vessels c.c.	Remarks
1	3·6	–	–	–	–	–	1·7	Not pregnant
2	2·7	–	7	–	–	–	0·6	"
3	3·4	–	10	–	–	–	1·6	"
4	3·1	–	4	–	–	–	0·6	"
5	2·3	10	15	5	5	(–)	1·65	
6	2·2	11	12·5	8	7·5	(–)	4·00	
7	2·5		36	6	335	–	5·2	Clots in uterus
8	3·9	12	25	6	11	(–)	9·0	
9	2·75	22	92	11	106	(–)	11·2	
10	2·5	27	68	6	121	–	10·0	Oper. during birth
11	2·7	27	78	9	289	(–)	13·8	Clots in veins
12	3·5	28	75	8	321	(–)	20·0	
13	3·1	26	92	9*	360	(–)	26·8	
14	2·5	30	76	10	335	–	Bleeding in the uterus	
15	3·7	30	45	5*	170	–	21·0	
16	3·7	30	77	11	312	–	29·0	
17	3·4	27	69	10	283	48	21	
18	3·4	30	82	8	207	39	11	
19	3·0	28	95	8	232	41	13·4	
20	3·2	27	91	11	376	(–)	36	
21	3·1	16	36	9	8	(–)	2·7	
22	3·3	29	49	9	222	24	8·8	
23	3·05		–	6	340	–	6·2	1 day after birth
24	3·25	–	–	6	374	–	9·64	"
25	2·20	–		6*	7	–	10·9	
26	2·25		–	7	135		14·1	
27		28?		8	48	–	12·5	
28	2·90	30	–	4	195	–	8·5	Just before birth

* Plus one rudimentary fœtus.

The principal finding was however of a somewhat negative character —only one correlation could be made out and that was of a very rough nature. It was a general relation between the quantity of blood in the uterine[1] vessels and the total weight of the fœtuses. This correlation is shown in Fig. 2 A which includes experiments in which the number of

[1] Unless otherwise stated the word uterine in this paper is used to include the whole female reproductive tract.

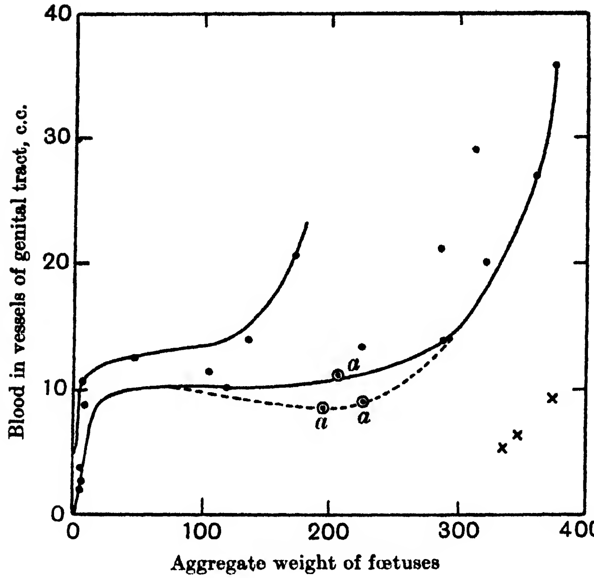

Fig. 2 A.

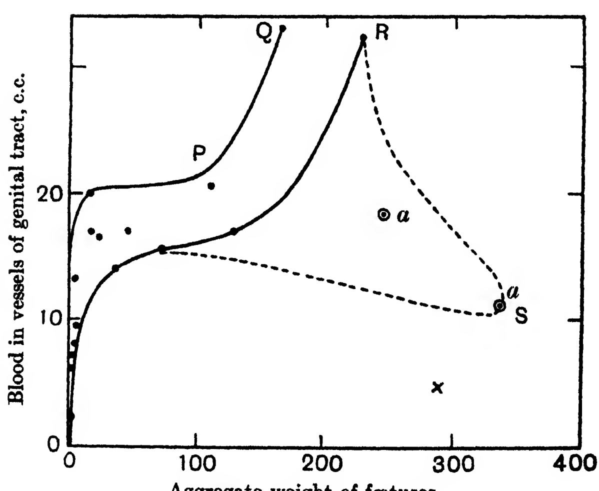

Fig. 2 B. For explanation of lettering see text.
Crosses = post-partem observations.

fœtuses have been from 6 to 11. When plotted a fairly definite sequence of relations comes out:

(1) The quantity of blood increases from about one to about 10 c.c. while as yet the weight of the fœtuses is insignificant.

(2) The weight of the fœtuses increases greatly in proportion to the quantity of blood.

(3) In the last phase the quantity of blood again rises relatively.

Towards the end of pregnancy there are 20–30 c.c. of blood in the uterus, but the day after parturition (the crosses in Fig. 2 A) the blood has very largely disappeared.

The bulk of the blood in the third stage is not actually in the uterus but in the great veins leading from it.

Series 2.

The experiments in series 2 were carried out by a technique evidently better than that employed in series 1, and which differed from the earlier methods in the following way:

Firstly, we were fortunate in being able to get in touch with a very reliable supply of rabbits, mostly Chinchillas. All the animals were

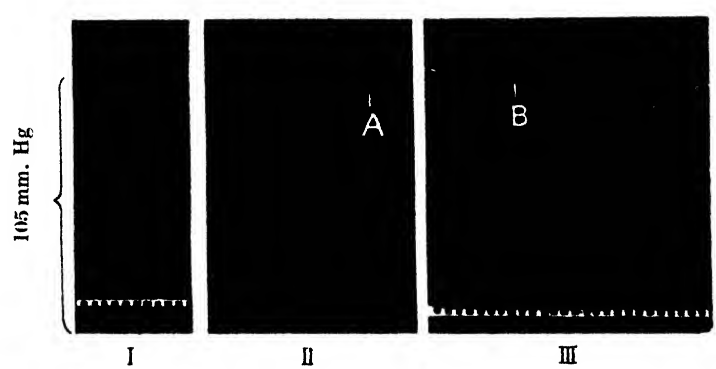

Fig. 3. General arterial pressure during certain periods of the dissection. Base line = bottom of black area. Time = seconds. I. at commencement. $p = 105$ mm. Hg. II A, insertion of ligature round vagina. III B, simultaneous tightening of all ligatures.

hardy, of very uniform weight and very carefully and reliably "stocked." A batch of six was stocked for us every week while we were at work, and very few rabbits failed to become pregnant.

Secondly, we were able to carry out the dissection and placing and tying of the ligatures without making more than a small local opening in the abdominal wall, so that in some cases the vessels had all been tied

up before we even saw whether or not the uterus was pregnant. Fig. 3 shows a tracing of the arterial pressure in such an experiment. The pressure was practically as high at the moment of tying the vessels as at the commencement of the experiment.

Thirdly, the experiments were carried out under dial, usually 2 c.c. injected into the abdominal cavity. Occasionally in addition a little C.E. mixture was given at the commencement of the experiment.

Fourthly, the hæmoglobin solution obtained from the uterine blood was filtered through Kieselguhr, a suggestion made to us by Dr Keilin. If about 10 c.c. of the filtrate are allowed to go to waste, the fluid which comes subsequently appears to have lost none of its hæmoglobin, whilst it is perfectly clear.

The data obtained in series 2 are given in Table II.

TABLE II.

No. of exp.	Wt. kg.	Days since impreg-nation	No. of fœtuses	Wt. of fœtuses or young g.	Wt. of fœtal placenta g.	Blood in uterine vessels c.c.
I	3·0	21	8	34·3	.	14·0
II	2·9	20	6	21·6	.	16·6
III	2·6	15	8	5·0	5·0	9·6
IV	Died 3·0	15	.	–	–	–
V	0·3	23	8	72·0	18·6	15·1
VI	2·8	22	7	42·0	14·0	17·2
VII	3·0	25	10	16·8	23·3	20·0
VIII	2·9	24	10	110·0	23·3	20·5
IX	2·7	26	5	127·5	16·5	16·7
X	2·9	25	0	–	–	2·6
XI	2·3	6	+	?	?	2·6
XII	2·4	6	+	?	?	3·0
XIII	3·7	15	11	3·3	7·2	?
XIV	3·1	29	8	236·0	20·5	32·3
XV	3·0	28	6	174·0	15·0	33·2
XVI	3·0	15	7	3·5	8·8	8·0
XVII	2·5	30	0	–	–	2·1
XVIII	3·1	29	0	–	–	2·1
XIX	Died on table	11	–			
XX	2·55	11	9	0·15	1·45	6·2
XXI	2·6	11	–	+	+	7·2
XXII		20	8	17·5	20·0	17·0
XXIII	2·9	30	9	337·0	22·5	11·4
XXIV	Died under dial	30	0			
XXV	2·4	30	6	290·0 (young)		
XXVI	3·3	30	0			
XXVII						
XXVIII	2·7	28	11	585·0 (young)	–	13·2
XXIX	3·0	30	7	245·0	21·0	18·4

This series was as fruitful in correlations as the previous one had been barren of them. The first to which we would draw attention is that

between the progress of the pregnancy in days and the quantity of blood in the uterus. This correlation is given in terms of the actual experiments in Fig. 4, whilst Fig. 5 gives the average obtained by taking the mean of the groups of black spots joined by dotted lines in Fig. 4.

The points to be noted are:

(1) That the maximum quantity of blood in the uterus is about 30 c.c. or perhaps $\frac{1}{8}$ of that in the rabbit.

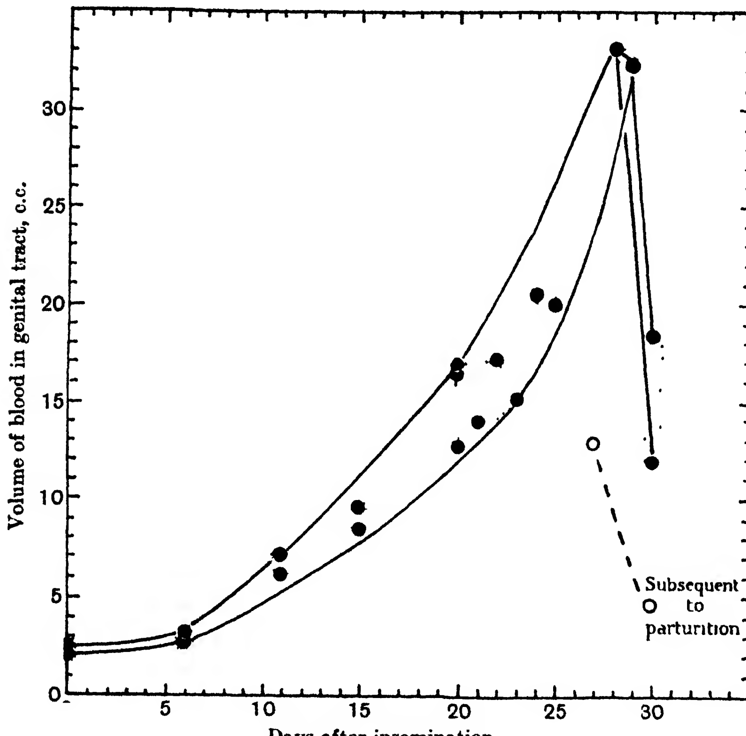

Fig. 4.

(2) That the vascularity of the reproductive tract commences to increase very early; certainly from the sixth day there is an appreciable rise in the volume of blood.

(3) That the peak is about the twenty-seventh to twenty-ninth day.

(4) That the volume of blood in the reproductive tract falls suddenly before parturition takes place. In this connection reference may be made to certain experiments in Table I. In experiment 10 labour commenced when the animal was on the table, though the record shows the

day as only the twenty-seventh. There were but 10 c.c. of blood in the uterus. Again in experiment 28 (Table I) the operation was on the thirtieth day and the contraction of the vagina suggested the imminence of labour; there were but 8·5 c.c. of blood in the reproductive tract.

The large drop in the quantity raises some interesting points in the consideration of which it is well to remember that we know nothing as

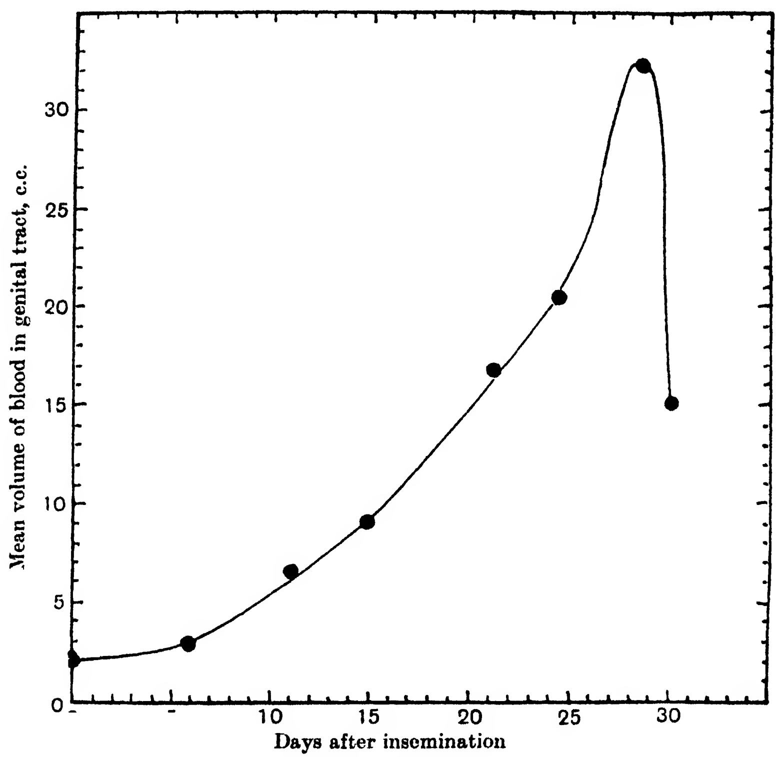

Fig. 5.

yet regarding the quantity of blood which circulates through the reproductive tract in a given time, as opposed to the quantity to be found in the uterine organs. It seems likely that the presence of 30 c.c. of blood is unnecessary either to the contractions of the uterus or to the actual life (over short intervals of time) of the fœtus. The question therefore may be asked what function so great a quantity of blood subserves in the uterine parts, especially as it is mainly to be found not in the organs themselves but in the large venous arcades and their

related veins. This question we cannot at present answer, but certain observations may be relevant.

Firstly, we have observed on more than one occasion that any undue pressure on the principal vein was associated with, and probably caused extensive intra-uterine hæmorrhages, on the same side as the venous obstruction. On one occasion there was some bleeding during the dissection from a little vessel in the region of the letter *C*, Fig. 1. The bleeding was stopped by pressure and the pressure involved the main uterine vein, but it was of short duration; on a second occasion when a similar accident occurred, care was taken never actually to stop the flow of blood in the uterine vein though doubtless there was sufficient pressure somewhat to raise the resistance to the passage of the blood. It is possible therefore that the copious system of anastomosing veins may be to prevent accidental pressure from raising the resistance locally and so producing intra-uterine hæmorrhages. The size and number of the embryos in the rabbit suggest that pressure on the veins is a real danger. This exaggeration of venous development in the mother is in great contrast to the calibre of the umbilical vessels. The naked-eye impression is that the sum of the sectional areas of all the umbilical arteries and veins would be less than that of any one of the larger veins which drain the uterus. One supposes that, at a late stage of pregnancy, the metabolism of the embryos constitutes all but a relatively small portion of the whole metabolism of the genital tract. If that inference is correct the mere exchange proper to the tract does not seem to demand so great a vascular development. The suggestion, however, that the main exchange in the uterine area is that proper to the growth and activity of the fœtal tissue is conjecture; we do not know whether work on a large scale is carried out in the placenta.

The great vascularity may be an index of some factor connected with the growth of the fœtus, as opposed to its mere existence. Other questions which at once arise are, What causes this pre-partural cutting down of the blood in the genital organs? Is the loss of blood a link in the chain which ultimately ends in labour? The answer to these questions we do not know.

Another correlation which we have observed is that between the progress of the pregnancy and the growth of the fœtus. This matter has already been the subject of observations as yet unpublished by Hammond [1931]. Hammond has very kindly shown us his figures of which ours afford a general confirmation (not that this is necessary); see Fig. 6.

A third correlation which we believe to be new is the relation in time between the vascularity of the uterus and the growth of the embryos. This is also shown in Fig. 6, depicting not only the weight of fœtal tissue expressed as a function of time, but also the parallel increment (over and above the normal 2 c.c.) of blood in the generative tract.

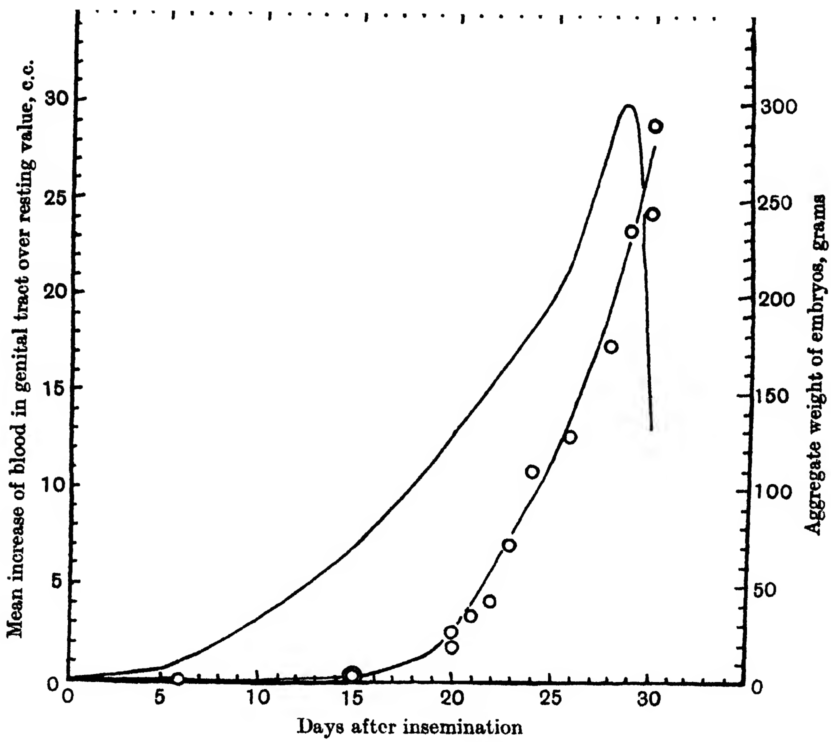

Fig. 6. Circles show the weights of the embryos. The line, without circles, the increment of blood.

The outstanding facts are that, whereas on the twentieth day the embryos are still of almost negligible bulk, the blood present has already attained to half its maximal volume, and indeed to a volume as great as on the thirtieth day.

An attempt to go further and correlate the blood volume in the maternal organs with either the number of embryos or the average weight of each embryo is less successful. On the whole on any particular day the more embryos there are the heavier their aggregate weight

appears to be (this is also a confirmation of Hammond's observations), but the proportion is not simple.

As a first approximation, though perhaps not in the final analysis, it seems fair to say that, on any particular day, the blood volume is almost independent of the number of embryos whilst the aggregate weight of the fœtuses is dependent on, but not a simple multiple of, the number.

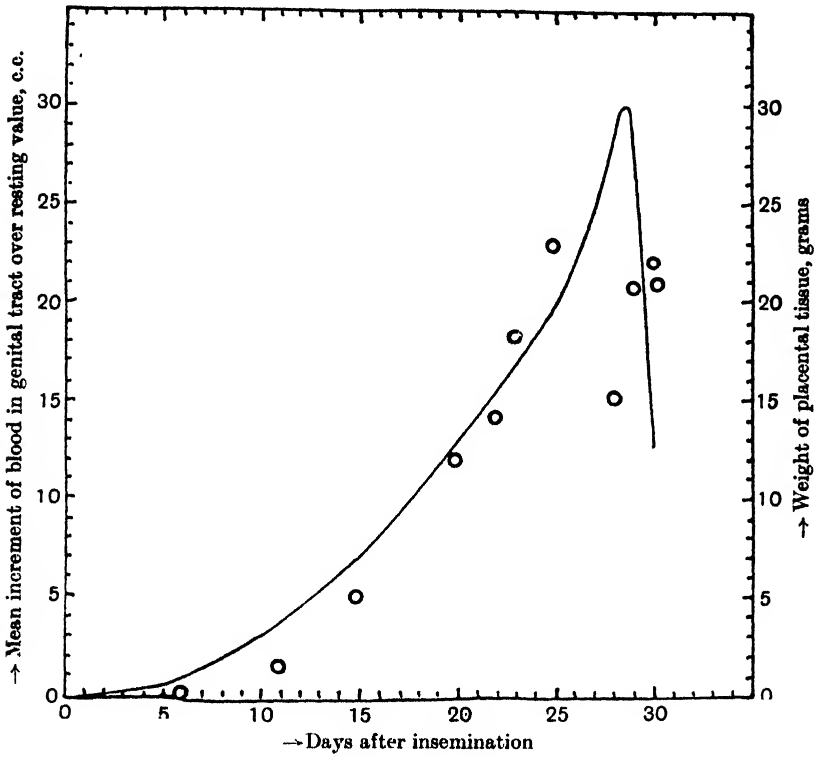

Fig. 7. Circles indicate the weight of the placentas. The line indicates the increment of blood.

We are now in a position to interpret Fig. 2. First let us consider Fig. 2B, in which the second series of experiments is plotted. We see that the first phase represents the first fortnight or so of the pregnancy in which the blood volume is increasing, while as yet the embryos are of negligible weight. The second phase represents the period from about the sixteenth to the twenty-fifth day, when the rate of fœtal growth is great proportionately to the increase in the blood. That this portion should be nearly horizontal is not surprising. More difficult matters are the

steepness of the final limb of the curve *PQRS*. Why in fact does the top of this portion stretch so far to the left as at *Q* and the bottom so far to the right as at *S*? Taking these points in order, the width of the distance *QR* is due to the fact just stated, that the maximal quantity of blood occurring about the twenty-eighth day will be about the same (30 c.c.) whether there be six embryos or eleven, but the weight of embryos in aggregate will be very different. (See Table II, exps. XIV and XV.) The position of *S* so far to the right is due to the measurements taken shortly before labour when the blood volume has fallen but the weight of the embryos is at its maximum.

To pass now to Fig. 2 A in the light of what we have learned from series 2. It is not surprising that the points marked *a* should indicate so small a volume of blood, they are from rabbits observed at a very advanced stage of pregnancy, two being on the thirtieth day and one on the twenty-ninth. (Rabbits are sometimes born as early as the twenty-seventh day.) The litters are all rather light, the points in question might easily be anywhere up to 400 g. with blood volumes of the same order. In Fig. 2 A and B the continuous lines enclose all the points where the blood and volume is increasing or maximal, the dotted line includes all points whatever.

The only correlation on the fœtal side which appears to follow the volume of blood in the maternal organs is the aggregate weight of the combined fœtal placentæ, so far as they can be detached from the maternal portions, and this correlation is very close up to the twenty-fifth day, but we have not obtained any growth between the twenty-fifth and twenty-eighth days (Fig. 7) in the fœtal placentæ which is commensurate with the peak in the maternal blood.

Summary and conclusions.

1. A technique is described for the measurement of the quantity of blood in the genital tract of the female rabbit.

2. During the resting condition the genital organs contain less than 2 c.c. of blood. They become appreciably more vascular from the fifth or sixth day onwards, and about half-way through pregnancy contain about 10 c.c. of blood. Up to this point the embryos are of negligible weight. By the twentieth day the embryos weigh only about 5 g. and the generative tract of the mother contains about 15 c.c. of blood.

The maximal quantity of blood, about 30 c.c., seems to occur about twenty-eight to twenty-nine days and the quantity falls rapidly before parturition. The significance of the arrangement of vessels is discussed,

especially in reference to the possibility of intra-uterine hæmorrhage due to pressure on the veins.

3. The quantity of blood in the generative organs on any particular day does not bear so near a relation to the number of fœtuses as does the total weight of the embryos.

4. The quantity of blood in the genital organs seems to be closely related to the combined weights of the fœtal placentas up to the twenty-fifth day when the latter seem to cease growing.

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OBSERVATIONS ON THE PROXIMAL PORTION OF THE EXTERIORIZED COLON.

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In previous papers it has been shown that the exteriorized spleen [Barcroft and Stephens, 1927] and loops of the small intestine [Barcroft and Robinson, 1929] of the dog yield valuable information concerning the function and activity of these organs under normal environmental conditions. Consequently it was of interest to study in a similar manner the activity of the exteriorized cæcum and proximal portion of the colon.

Although there may be some doubt as to whether the activity of the cæcum and colon under these conditions is exactly normal, we believe that our results are fully as valuable as those obtained by such methods as opening the abdominal cavity and surrounding the intestines with warm saline [Alverez and Zimmerman, 1927], or inserting balloons into the lumen of the intestine and recording movements on a smoked drum, as originally done by Bayliss and Starling [1901] and more recently by Templeton and Lawson [1931], or even the still more satisfactory method of Cannon [1902], who X-rayed the intestines after the administration of a barium meal. This last method, no doubt, has an advantage over the other two in that the animal can be observed under normal conditions, and that anæsthetics need not be given nor the animal sacrificed; but it too has its disadvantages in that the observations are indirect and made not on the gut itself but on its contents. Also the weight of the barium in the intestine may interfere with the normal movements.

Our method, we believe, though it has its own disadvantages, may add to the knowledge already obtained by the X-ray method in that (*a*) we can make observations on the intestinal muscles themselves rather than on their contents; (*b*) we study the animal, not under anæs-

thetic or during an operation, but after the animal has returned to normal health and activity; (*c*) we can study the same animal over a long period of time under various conditions, such as starvation, response to foods, and the effects of various drugs, such as morphine and magnesium sulphate, and their relation to the water content and movements of the colon.

Our data represent observations on three dogs. Before presenting the data, however, we consider it important to give an account of the operation and the time and progress of the dogs in their return to apparently normal activity. In each case a female dog was used, and no food was allowed for a day before the operation. One-half c.c. of 5 p.c. morphine was injected 1 hour before the operation. The anæsthetic used was an ether-chloroform mixture.

The steps of the operation were as follows: a transverse incision was made through the skin and abdominal wall about 1 inch above the umbilicus, extending about 2 inches on either side of the midline. At each end of this incision we cut away a circular piece of the abdominal muscle wall so as to avoid undue constriction of the ileum at one end and the more distal colon at the other. Then an opening of about the same size was made in the omentum and the colon located. The colon is usually found rather deep in the abdominal cavity and just below the lower curvature of the stomach, and may be recognized by the longitudinal striations of its muscular wall, which are more pronounced than those of the small intestine. After it was found and drawn to the outside, the cæcum was uncoiled slightly and anchored to the outside of the abdominal wall with one or two sutures. A number of catgut sutures sufficient to pull the abdominal wall together underneath the exteriorized colon were then put in place but not tied. Following this, other sutures were put through the skin in the same manner. Then, while the colon was held up in position, the muscle sutures were drawn up and tied, and then those of the skin. Care was always taken in placing the sutures to avoid constriction of blood vessels underneath the exteriorized portion, and at the same time, not to draw them too tightly. The total length of the exteriorized portion was about 12 cm., of which the cæcum made up 2½ cm.

After the operation was completed, the exposed portion was smeared liberally with vaseline, and supported on either side by a thick pad of cotton wool. These pads were held in place by a bandage wrapped tightly around the animal's abdomen. Clean bandages were put on every other day for at least the first 2 weeks, and the area washed with warm

saline, except when any sign of infection occurred, at which Eusol was substituted. The animals were kept in a warm room at a temperature of about 28° C. for at least a week after the operation, and to keep them quiet small doses of morphine were occasionally injected during the first 2 days. The rectal temperature was recorded daily. In regard to food, the animals were given water, usually containing glucose, on the second day; on the third day they were allowed both water and diluted milk, and on the fourth a small quantity of boiled fish. This fish diet was continued for nearly 2 weeks before more solid food was given.

The first fæces in each case were found in the cage on the morning of the fifth day, this being coincident with and probably related to an increasingly solid diet. As was expected, the fæces were of a very watery consistency, which continued from 10 days to 2 weeks and then gradually became more concentrated. To obtain information on the rate of concentration of the fæcal material from this time on, the water content was determined by collecting two samples of fæces immediately after defæcation, in weighing bottles, and drying them at 100° C. The rate of concentration of the fæces of the three dogs and the number of days required to reach a constant concentration are recorded in the following table.

TABLE I. The rate at which fæces concentrate after the operation.

Dog I—"Susie"		Dog II—"Florence"		Dog III—"Nina"	
Days after operation	H_2O of fæces, av. 2 samples (p.c.)	Days after operation	H_2O of fæces, av. 2 samples (p.c.)	Days after operation	H_2O of fæces, av. 2 samples (p.c.)
17	77·8	15	84·8	19	84·6
19	70·0	22	79·0	24	80·2
26	64·7	27	72·4	31	72·0
52	61·9	34	71·3	80	71·7

As Table I indicates, within 12 days after the fæces were noticed to be less watery the water content of the fæces had reached a level, this level being determined by comparing the last samples with some taken at a random time after apparent recovery of the dogs.

In conjunction with this watery state of the fæces, it was noticed that there was very little sign of activity in the muscular walls of the colon, and also that the area appeared to be congested and inflamed. It is possible that this condition was due to a loss of muscular tone brought on by the manipulation of the colon during the operation. But as the colon gradually took on a more healthy colour, a series of small kneading contractions were detected; at the same time the colon appeared to

become more distended, and soft and flabby to the touch. This state of recovery gradually spread back toward the cæcum, which regained its tone and normal colour about a week after the colon. The interesting fact in this connection is that at the time the colon and cæcum were regaining their tone and activity the fæces were becoming more concentrated, which is quite in keeping with the idea that the proximal colon and cæcum are closely related to the concentration of the fæces.

Another point of interest is the manner in which the act of defæcation was carried out, particularly by the dog "Susie," before and after the activity of the colon had been re-established. Previous to the recovery, it was noticed on many occasions that, whenever the dog was about to defæcate, she would run around the room in a distressed manner and at intervals slow down her pace and shake her hind quarters and tail most violently. This act she repeated four or five times, and then, without even squatting, would pass a thin watery mass of fæces with considerable force. But after the tone and activity of the colon and cæcum had been regained the act of defæcation was carried out quite normally. We also observed this same kind of behaviour on the part of the dog later when we were studying the activity of the colon after the administration of morphine.

These observations lend evidence to the view advanced by Hertz and his collaborators [1907] that, just previous to the act of defæcation, the proximal colon and cæcum contract, forcing the fæcal matter down into the rectum. Up to the present it has been impossible to observe the colon during actual defæcation, but we have observed it just before and after the act and found that the colon is very much distended before and emptied after. It has also been noted on several occasions that if the colon was kneaded for any length of time and the fæces thereby pushed along toward the rectum, upon releasing the dog, the act of defæcation took place.

Within 3 weeks after the operation it was noticed that an epithelial layer, starting from the cut edges of the skin, was growing over the exteriorized portion, and finally covered it completely. This is the same type of protective layer as covers the exteriorized spleen (Barcroft).

In discussing the types of movements found in the colon and cæcum, one is confronted with the difficulty of finding a satisfactory means of presenting the data. Moving pictures would be ideal for demonstration, but impractical for publication. Graphic records, although convenient, present mechanical difficulties in representing the true activity of the organ and are often misleading. We concluded, therefore, that direct

observation with the eye and a descriptive tabulation of the data were the most convenient and satisfactory for our purpose.

As any extraneous noises disturbed the dogs, we carried on our observations in a practically soundproof room. The animal was studied lying on her side on a table, the time of observation often lasting 1½ hours. We made observations of movements occurring (*a*) from ½ to 12 hours after feeding, and while feeding the dog on the table to note the presence of any gastro-colic reflex, (*b*) before and after giving her 2 g. of magnesium sulphate in 150 c.c. of water by way of stomach tube, and (*c*) under the influence of morphine. Since these three types of conditions produced different effects we shall treat them separately. The following discussion and figures will represent the findings on the two dogs "Susie" and "Florence." All the types of movements found were confirmed in the third dog, but not to such a pronounced degree, due to improper healing of the epithelium of the colon.

(*a*) *Normal movements of the cæcum and colon.*

The type of movements most frequently seen in the cæcum and colon occurred ½ to 12 hours after the animal had been fed. These movements started in the cæcum, where slight but definite kneading contractions occurred in the lower end, appearing three or four times, at intervals of about ½ minute. These were followed by a deeper contraction which involved practically the whole cæcum and usually had a forward movement, pushing on down toward the ileo-cæcal region. Then there was a tendency for the cæcum to relax, but shortly after, another deep cæcal contraction appeared, and often a third and fourth. They sometimes followed one another so closely that the cæcum appeared to relax hardly at all.

By this time there was usually a marked constriction in the region of the ileo-cæcal valve which remained contracted for 3 or 4 minutes. As a result of these cæcal contractions the colon became quite distended, and shortly afterwards one might see shallow anti-peristaltic waves appearing near the region where the colon entered the abdominal cavity. They continued to course back toward the ileo-cæcal constriction at a rate of five to seven a minute, becoming deeper and deeper as they continued along the colon. By this time the cæcum and ileo-cæcal constrictions were usually beginning to relax. After these anti-peristaltic waves had coursed back for about 2 to 4 minutes, a series of deep kneading contractions, about 1 cm. apart, appeared to start near the ileo-cæcal region, and work on down the colon, stopping the progress of the anti-peristaltic

waves. In some cases, by the time these kneading constrictions had reached the distal end of the colon, the ileo-cæcal region had become quite relaxed, while in other cases the whole colon seemed segmented at one time.

A second type of movement which we observed frequently had its origin in the ileo-cæcal region. It was a deep massive contraction that nearly occluded the lumen of the colon, and then very slowly passed on down the colon, forcing a mass of intestinal contents before it. As this

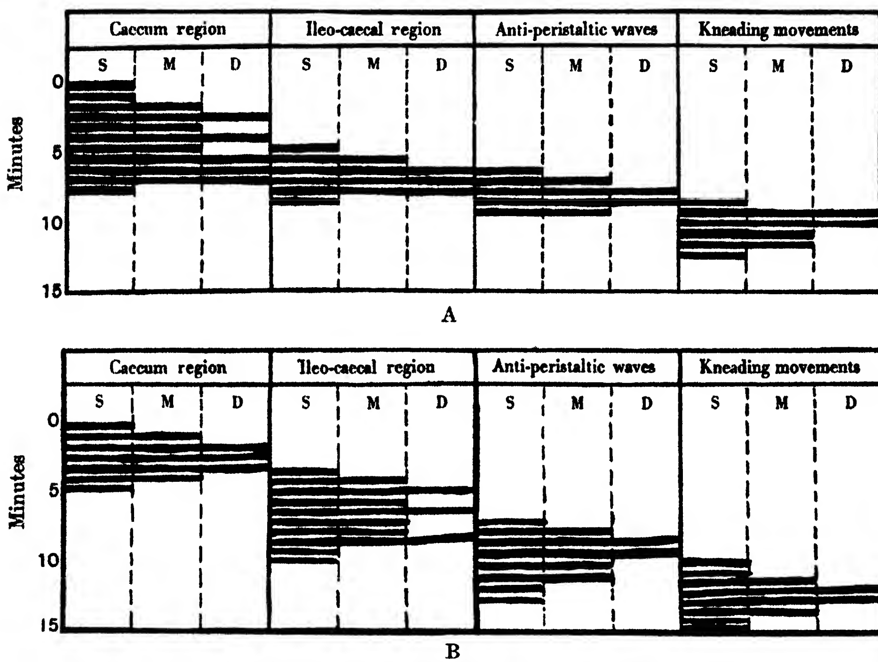

Fig. 1 A and B. The normal type of movements found in the cæcum and colon in dogs "Susie" and "Florence" respectively. S. Shallow contractions. M. Medium contractions. D. Deep contractions.

contraction moved along, there appeared in the distended portion of the colon just ahead of the contraction, about 1 cm. in length, a group of hardly perceptible anti-peristaltic waves. The total time it took the contraction to travel the length of the exposed colon was about 15 minutes. It seemed to occur when the fæcal content was rather dry and solid, for on no occasion was it observed when the colon was well distended with a watery fæcal material.

Quite different from these results were those obtained directly (2–5 min.) after the dog was fed with a biscuit or two on the table. In

this case the contractions first occurred in the ileo-cæcal region and colon, and not in the cæcum as before. These constrictions became very pronounced and were usually followed first by the anti-peristaltic waves and

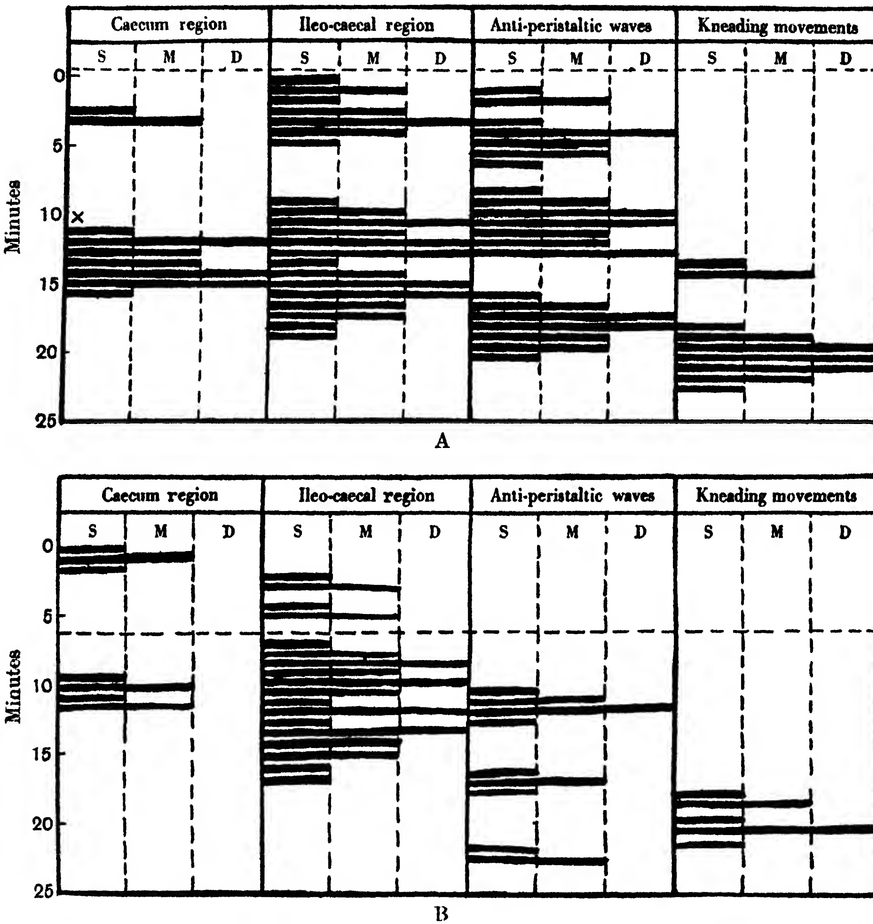

Fig. 2 A and B. Evidence of a gastro-colic reflex. The dotted horizontal line represents the point at which food was given under observation. (X) in Fig. 2 A is the point at which normal series of movements appears. (See Fig. 1.)

then by occasional kneading contractions. Then, with short periods of slight relaxation between constrictions, this series of movements was repeated again and again for about 10 minutes. The cæcum all this while remained comparatively inactive, in some cases for 30 minutes after the food had been given.

This type of contraction corresponds to those described by Hertz and Newton [1913], who, using X-rays on humans, found that similar movements occurred directly after the entrance of food into the empty stomach, and explained it as a gastro-colic reflex to clear the colon of material for the entrance of further intestinal content from the small intestine.

To present these two types of intestinal movements in a graphic form we have prepared the preceding figures (Fig. 1 A and B and Fig. 2 A and B). In these graphs the lengths of the horizontal bars represent the depth of the contractions, their positions on the vertical line indicating the times at which they appear. However, the number of bars does not indicate the number of contractions which occurred; the graph is designed to show the intensity of the movements and not the quantity.

Fig. 1 A and B give examples of the normal movements, and show how the cæcal movements appear first, followed by those in the ileo-cæcal region and then those in the colon. This graph does not include a representation of the slow progressive movement in the colon which we have referred to as the "second type." Fig. 2 A and B represent the gastro-colic reflex shortly after the food has entered the stomach, the major movements in this case being contractions of the ileo-cæcal region and anti-peristaltic waves, with very little evidence of cæcal and kneading movements. At (×), in Fig. 2, however, it may be noticed that the cæcal movements again appeared, starting a series of normal movements like those in Fig. 1.

(*b*) *Effects of* $MgSO_4$.

Observations were made on the colon and cæcum before and after giving 2 g. of magnesium sulphate in 150 c.c. of water by stomach tube. The results obtained can be best described by the use of Fig. 3 A and B.

As will be noticed, the usual cycle of movements appeared before the injection. As shown in Fig. 3 A, which represents the dog "Susie," within 15 minutes after the injection we found movements like those described in Figs. 1 and 2, the cæcum being very inactive and the other parts quite active. Three hours after the injection, however, the colon and cæcum were observed to be very much distended and less active, the only movements noticeable being shallow anti-peristaltic waves, appearing quite regularly and occasionally developing into deeper waves; and at times contractions of the ileo-cæcal region were also observed. The cæcum at this time was very much distended and practically inert,

and there were no kneading movements following the anti-peristaltic waves. About 5 hours after the injection, the movements were similar to those just discussed, except that there seemed to be more activity in

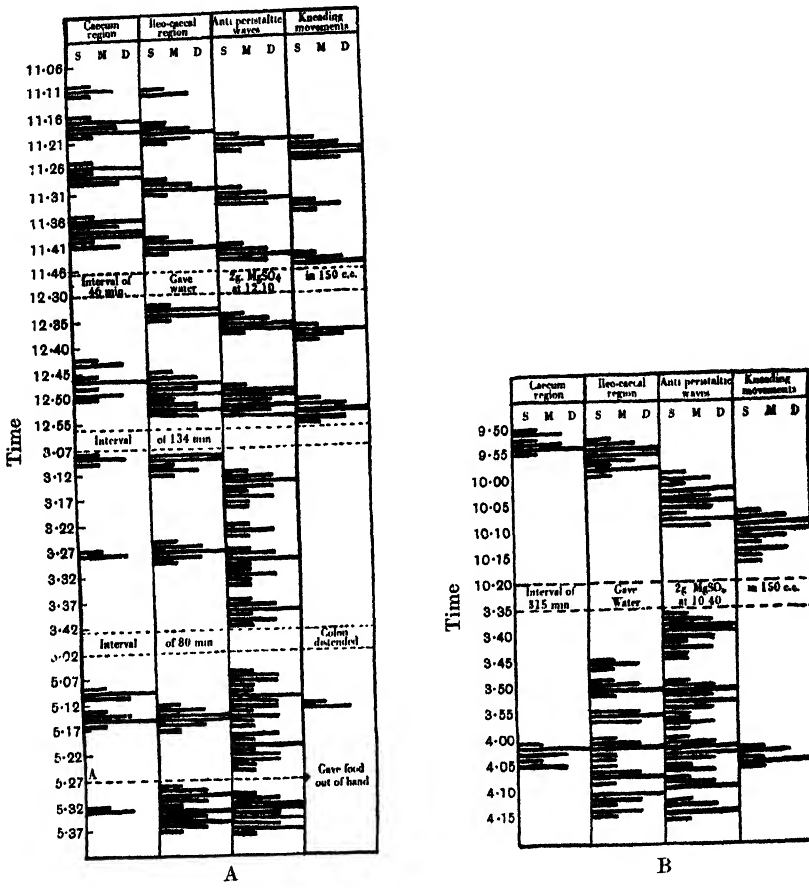

Fig. 3 A and B. The effects of magnesium sulphate by stomach tube with observations at different intervals of time after the administration.

the cæcum than before. With this dose of 2 g. of magnesium sulphate, defæcation took place from 5½ to 6 hours after its administration, the fæces being very watery, as would be expected. In one case the colon was observed directly after defæcation and was found to be empty and very inactive.

(c) *Effects of morphine.*

Since it is generally accepted that morphine has an inhibitory effect on the intestinal movements, and is often prescribed in cases of diarrhœa, it was of interest to observe its effect on the exposed colon. The dogs were injected with ½ c.c. of 5 p.c. morphine, and an hour later showed the customary symptoms, such as vomiting, defæcation and weakness of the hind legs. Observations made during the course of the day showed that the cæcum and ileo-cæcal region were the only portions of the gut to show any movement, and even these were very slight and occurred only occasionally. Very rarely a slight kneading movement appeared in the colon. Late in the day the colon became very distended. In no case was there any sign of anti-peristalsis or progressive movement in the colon. In one of our dogs ("Florence") the morphine seemed to cause a slight increase in the tone of the colon, an observation quite in accord with the recent work of Gruber and Robinson [1929] on the small intestine. This increase in tone probably accounts for the more frequent kneading movements in the dog.

Fig. 4 A and B are graphs of the observations made on the exposed colon after the injection of morphine.

Before any theoretical statement concerning the significance of these different movements in the dog's colon and cæcum is given, it may be said that the existence of anti-peristaltic waves in the dog's colon has hitherto been a subject of controversy. Cannon [1903] and Henderson [1909] are of the opinion that they do occur in the colon of the dog as well as the cat. Thomas and Kuntz [1926] also report a case in which they observed anti-peristaltic waves in the colon of a dog after giving large doses of nicotine. Elliott and Barclay-Smith [1904], although agreeing with Cannon concerning their appearance in the cat and other animals, were unable to detect any in dogs. Recently, however, Templeton and Lawson [1931], using the balloon method, recorded movements very similar to ours, and stated that they had found forward and backward movements passing along the colon of the dog, but hesitated to classify them as peristaltic and anti-peristaltic waves.

Just why there has been so much difficulty in finding these anti-peristaltic waves in the dog is hard to understand, since in our experiments we seldom failed to find them if the dog was observed for more than 20 minutes at a time. Furthermore, our observations on the ordinary cycle of movements in the colon of the dog are almost identical with those of Cannon on the cat, even in the rate of the waves and the

frequency of their appearance. What he refers to as a "marked bulging between successive constrictions following the deeper anti-peristaltic waves" we have called a "kneading" type of movement.

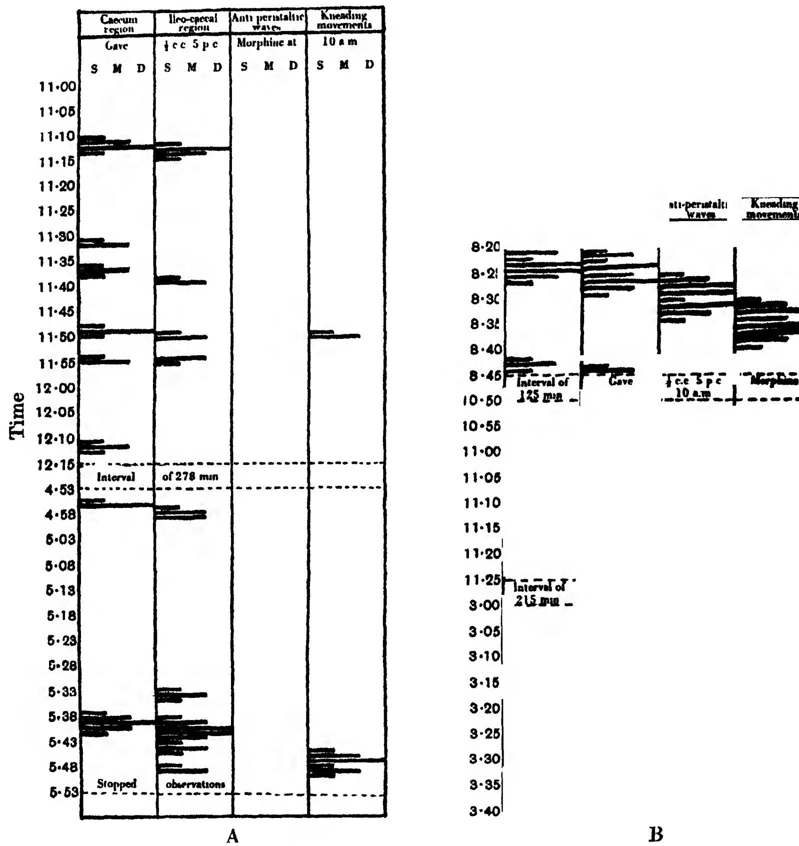

Fig. 4 A and B. The effects of morphine sulphate on the movements of the cæcum and colon at different intervals of time after subcutaneous injection.

The conclusions concerning the physiological significance of the movements we observed can be only theoretical and subject to criticism. But, judging from the consistency of their mode of action and the order of their appearance, we believe that the cæcum plays an important rôle in the concentration of the fæces. It also seems to be one of the functions of the colon, through its anti-peristaltic and kneading contractions, to churn and force fæcal material back toward the cæcum. The deep, slow, progressive wave along the colon, which has similarly been described by

Elliott and Barclay-Smith, we believe is the means of pushing the more concentrated material on down into the more distal colon, since it appears only when the material is quite concentrated, and was rarely noticed when the colon was distended with watery fæces after injections of $MgSO_4$.

SUMMARY.

1. From 2 to 3 weeks after the colon and cæcum had been exteriorized, the water content of fæces began to diminish along with the return of activity and tone of the exteriorized parts.
2. There is a typical series of movements, consisting of cæcal contractions, followed by anti-peristaltic waves and then a type of kneading contractions in the colon. These series usually occur at intervals of about 30 minutes.
3. There is evidence of an occasional slow, deep, progressive wave in the colon.
4. We found anti-peristaltic waves in the colon of the dog, appearing at the rate of five and seven a minute.
5. There is evidence of a gastro-colic reflex, appearing within a few minutes after feeding.
6. Movements were observed after the administration of magnesium sulphate and morphine, which differed from the normal in both frequency and intensity.

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612.146:612.59

THE OPTIMUM TEMPERATURE FOR INVESTIGATIONS ON THE FROG'S CIRCULATION.

By E. M. SCARBOROUGH.

(From the London (R.F.H.) School of Medicine for Women.)

In a previous paper [Cullis and Scarborough, 1932] attention has been drawn to the influence of temperature in modifying the effects of certain drugs on the frog's circulation. Preliminary experiments on the influence of variation in temperature only on the blood-pressure, heart rate and web circulation of the frog were also reported. Before proceeding to further experiments involving the use of drugs, it seemed advisable to repeat a series of experiments in which, as far as possible, temperature should be the only variable factor, in order to ascertain the optimum temperature for the investigation of drug effects on the circulation.

Method.

The experiments were carried out during the winter months (November to March, 1931-2). With a few exceptions male frogs of fairly uniform size were selected. A method which has been previously described [Cullis and Scarborough, 1932] was used, and observations were made on the blood-pressure, heart rate and web circulation, the temperature being kept constant throughout each experiment. The series consisted of five groups of twenty consecutive experiments. The temperature of each group was different. The temperatures used were 5, 10, 15, 20 and 25° C.

Results.

The duration of most of the experiments was 3 to 4 hours. The following results are from observations taken at intervals of ½ hour during the first 3 hours of each experiment. One experiment at 25° C. is omitted as the circulation failed within an hour.

The relationship between heart rate and temperature, which has been shown to be a linear one [Taylor, 1930], was maintained throughout

the series with three exceptions. These experiments (two at 20° C. and one at 25° C.) are also omitted. In each group the average heart rate at the end of 1 hour differed by less than 1 beat from the average rate at the end of 3 hours.

The relationship between blood-pressure and temperature was less obvious. The initial readings and the subsequent changes in blood-pressure varied considerably, and average figures do not fairly represent these variations. Fig. 1 shows the blood-pressure changes in five single experiments, one from each group, the experiment chosen being the one

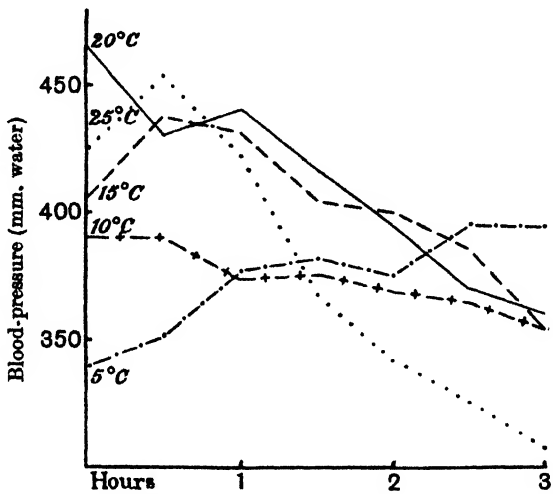

Fig. 1. Blood-pressure changes in single experiments at 5° C., 10° C., 15° C., 20° C., and 25° C.

which started nearest to the mean initial blood-pressure of the group. At 5° C. the blood-pressure rose during the experiments, with only two exceptions in which it fell. At higher temperatures the changes were more variable, but the blood-pressure showed a general tendency to fall throughout (at 10° C. there were two exceptions, at 15° C. seven, at 20° C. five and at 25° C. two in which the pressure was maintained or rose). The fall was most marked at 25° C. The initial readings were generally lowest at 5° C., and the final readings lowest at 25° C.

The web circulation was brisk and open (using "open" in reference to the number, not the calibre, of active vessels) at the onset of the majority of the experiments (80 p.c. or more at 5° C. to 20° C., 55 p.c.

at 25° C.). At the end of more than half of the experiments the web circulation was slow and closed (*i.e.* number of active vessels diminished). Fig. 2 gives a graphic representation of the condition in each group at hourly intervals throughout the series.

It will be seen that the web circulation failed most markedly at extreme temperatures, and only at 15° C. was well maintained for 3 hours. The web circulation was good in 72 p.c. of the observations in which the blood-pressure was above 350 mm. water and in 40 p.c. of those below. At 5° C. only a rise in blood-pressure was generally associated with impairment of the web circulation. In the few experiments in which blood-pressure rose at other temperatures the web circulation was

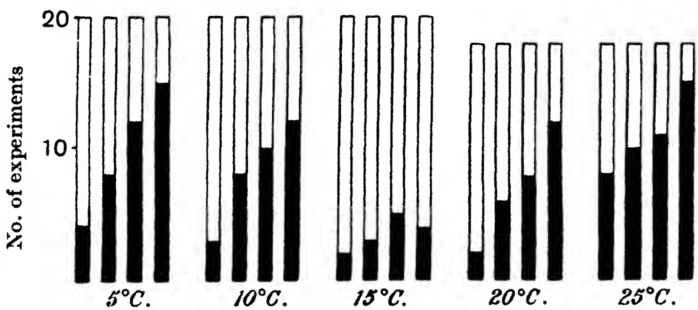

Fig. 2. Condition of the web circulation (white = good, black = poor) at different temperatures. The first column of each group represents the condition at the beginning of the experiments, and the second, third and fourth at the end of intervals of 1 hour.

maintained. At 15° C. a fall in blood-pressure was associated with a good web circulation, with two exceptions in which the fall was excessive. In experiments in which the blood-pressure fell at other temperatures there was a general tendency towards impairment of the web circulation. It is perhaps of interest to report that during the four experiments at 5° C. in which the web circulation was maintained the frogs struggled slightly. Such struggles occurred during three other experiments at 5° C. and occasionally at other temperatures. Throughout the series impairment of web circulation was associated with closing of the capillary network and slowing of the flow through the larger vessels. There was no marked change in the calibre of the active vessels, and no obvious difference between the impairment which developed at 5° C. and at 25° C.

The following results were obtained from experiments in which the frog's vessels were perfused with Ringer's solution at 15° C. and then at 5° C. or 25° C. At 5° C. the vessels were constricted in four out of six experiments; at 25° C. the vessels were dilated in four out of six. Direct application of water at 5° C. to the web caused closing of the capillary network in each of six observations, with slowing and congestion in the larger vessels in five out of six. Water at 25° C. caused transitory closing of the capillary network in three out of six observations, and no change in the larger vessels.

Discussion.

The blood-pressure is best maintained at 5° C., the web circulation at 15° C. The web circulation fails at 5° C. in spite of a rise in blood-pressure, and is maintained at 15° C. in spite of a fall. This result suggests that at 5° C. the blood-pressure is maintained or rises because the peripheral circulation is cut off. Brooks [1918] has shown that the leopard frog becomes very sluggish and inactive in water below 5° C. This inactivity, comparable to that of the hibernation period, may be associated with a similar circulatory change. The results of perfusion of vessels and direct application to the web indicate some active constriction at low temperature. Krogh [1920] found that local cooling produced dilatation in the lingual vessels, but [1921] that cutaneous capillaries differed from lingual in response to stimuli. At high temperatures the condition of the web circulation is largely determined by the actual height of the blood-pressure, and by the rate of fall.

The optimum temperature for the investigation of drug effects on various factors of the intact circulation is considered to be 15° C., given that occasional experiments are excluded in which the blood-pressure falls excessively and the web circulation is impaired. 15° C. is the temperature of the animal house in which the frogs are kept, and it is found that better results are obtained if the animals are acclimatized there for a few days before use. Further investigations are at present being made upon the effect of temperature on the absorption of drugs, and also upon the actual rate of absorption from the lymph spaces at 15° C.

Summary.

1. Results of experiments upon the influence of temperature on the circulation of the frog show (*a*) that the blood-pressure rises at 5° C., and tends to fall at other temperatures; (*b*) that an active web circulation is maintained best at 15° C.

2. It is suggested that the rise of blood-pressure at 5° C. is associated with an active constriction of the peripheral circulation.

3. 15° C. is considered to be the optimum temperature for the investigation of drug effects on the circulation.

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THE RÔLE OF LACTIC ACID IN NERVE ACTIVITY.

By T. P. FENG[1].

(*From the Department of Physiology and Biochemistry, University College, London.*)

GERARD AND MEYERHOF [1927] showed that nerve left in nitrogen produces lactic acid at a rate apparently unaffected by stimulation, and that the lactic acid formed during anaerobiosis is not removed on readmission of oxygen. Later Holmes, Gerard and Solomon [1930] found a slight rise in the lactic acid content of stimulated bull frog sciatics in oxygen, which, however, as the authors themselves pointed out, might really have been due to partial asphyxia on account of the close packing of the nerves. At any rate their parallel experiments on rabbits' nerves gave a slight decrease in the lactic acid content of stimulated ones. With this absence of a change in the lactic acid content there was also no change, as a result of stimulation, in the "free sugar" and glycogen. These results contrast strikingly with those found in muscle and make it doubtful whether lactic acid formation and oxidation constitute any part of the chemical events accompanying nerve activity. If, however, lactic acid forms no part of the working mechanism of nerve, it should make no immediate difference to the nerve whether its formation is suppressed or not. Poisoning, therefore, with iodoacetic acid furnishes a means of investigating the problem further.

The effect of iodoacetic acid was first roughly tested by soaking the nerve of a gastrocnemius-sciatic preparation in 1 p.c. iodoacetic acid (hereafter I.A.A.) with the muscle hung above the solution. After over 4 hours, crushing the nerve still called forth contraction. This at first gave the impression that nerve was practically immune to the action of I.A.A. The poisonous effect, however, was exposed by a study of the capacity of I.A.A. treated nerves for prolonged activity, employing the action current as indicator. Such nerves deteriorate progressively when tetanized and fail altogether in the course of 2 hours or so, depending on the degree of poisoning and the rate of stimulation.

[1] Tsing Hua University Fellow.

Method.

The sciatic nerves of medium-sized Hungarian *Rana esc.* were used throughout this work. In any one experiment two symmetrical nerves were employed, one serving as a control for the other. They were first soaked in the solution to be studied and then mounted in the ebonite chamber sketched in Fig. 1. The chamber was separated into two compartments (left and right, Fig. 1) by a wall provided with two slots, which accommodated the nerves and could be sealed when desired by vaseline. Each compartment was divided down the middle by an incomplete partition represented by the dotted line in Fig. 1. This parti-

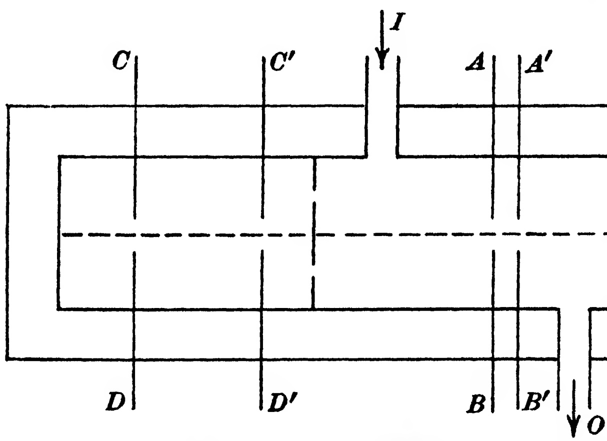

Fig. 1. Ebonite nerve chamber. *I*, gas inlet; *O*, gas outlet; *AA′*, *BB′*, stimulating electrodes; *CC′*, *DD′*, action current electrodes. See text for further description.

tion increased the surface for holding wet filter paper so that the nerves could be more easily kept moist, and helped to avoid electric leaks from one set of electrodes to the other. Electric insulation was further secured by coating the interior of the whole chamber with paraffin wax.

AA′ and *BB′* are stimulating electrodes; *CC′* and *DD′* are leads to a Downing galvanometer with coils of total resistance about 20,000 ohms, the resistance of the nerve between the galvanometer leads being of the order of 70,000–80,000 ohms. The working sensitivity was about 1 mm. = 5×10^{-11} amp. A condenser of 20 mfd. capacity was in series with the galvanometer to block the injury current. Continuous stimulation was given in most experiments by an induction coil with a rate of about 50 make and 50 break shocks per sec., sometimes by repetitive condenser charge and discharge with the aid of a commutator. Test stimuli

were always by induction coil, tetanizing for a time sufficient for the galvanometer deflection just to reach its maximum, usually about 1 sec.

Apart from chemical determination of lactic acid formation there is no known reliable indicator of a sufficient degree of I.A.A. poisoning in nerve such as in muscle is afforded by the onset of contracture. After a number of trials concentrations around 0·4 p.c. were adopted as suitable. Such concentrations are well below that which may interfere with respiration [see Gerard, 1931] and give a striking poisonous effect to be described presently. Whether they inhibit the formation of lactic acid has not been determined chemically. It was found that for sure poisoning the duration of soaking must not be less than 2 hours. This is not astonishing since the connective tissue sheath of frog's nerve is a very efficient diffusion barrier [see Feng and Gerard, 1930], and I.A.A. poisoning is progressive, requiring some time to develop.

Results.

(1) *The rapid failure of* I.A.A. *poisoned nerve when stimulated in oxygen.*

The most conspicuous sign of I.A.A. poisoning in nerve is the inability of such nerve to endure prolonged activity. Fig. 2 plots galvanometer deflection, representing action current to a test stimulus, against time of tetanization. Curve *A* was given by a normal nerve; *B* by the symmetrical nerve previously soaked in 0·4 p.c. I.A.A. for 3 hours. The I.A.A. nerve failed completely after 1½ hours' continuous stimulation, while the normal nerve gave even a larger response after the same amount of stimulation. That failure is somehow brought about by stimulation and is not due merely to the gradual development of the I.A.A. effect is clearly shown by the steep fall of curve *B*, shortly after the application of continuous stimulation, from a slightly rising base line.

Assuming that with the concentration employed the above recorded effect of I.A.A. is entirely related to the inhibition of lactic acid formation, the rapid failure of stimulated I.A.A. nerve may be attributed (*a*) to the absence of formation of lactic acid with its attendant energy liberation, and (*b*) to the loss of the free energy that comes from the oxidation of the lactic acid formed. Probably both (*a*) and (*b*) are contributory to the deleterious effect of I.A.A., but they can be separately tested (i) by studying the performance of I.A.A. nerve in nitrogen and (ii) by supplying I.A.A. nerve with lactate.

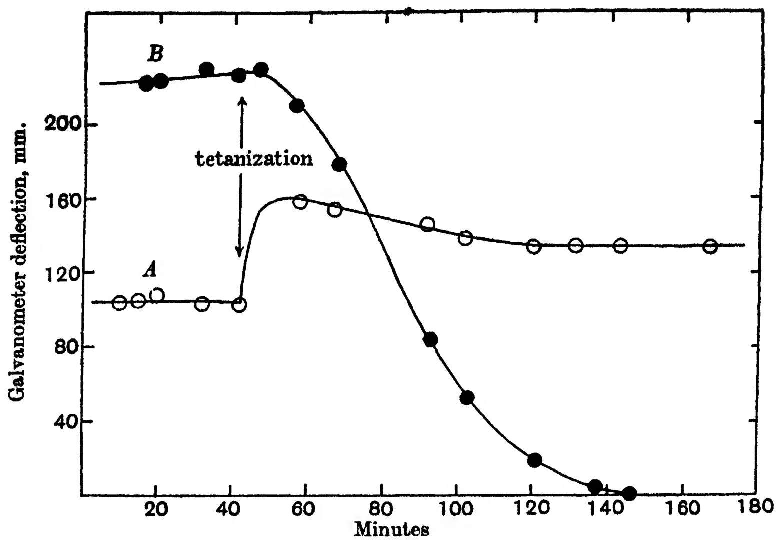

Fig. 2. The effect of continuous tetanization on the response: *A*, normal nerve; *B*, I.A.A. poisoned nerve: both in oxygen. The points plotted are galvanometer deflections representing action current to a test stimulus of about 1 sec. duration given by induction coil during a short gap in the tetanus. Note that *B* failed completely after about 100 min. tetanization, while *A* gave a greater response after the same amount of stimulation.

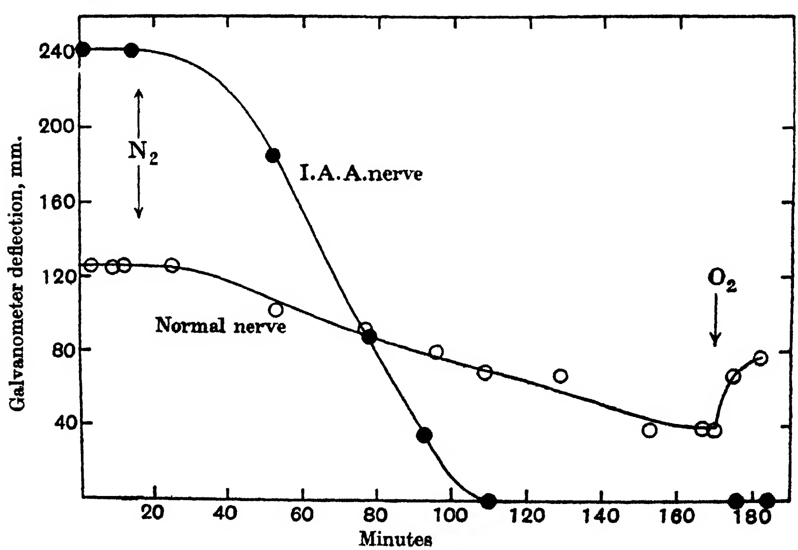

Fig. 3. The rate of asphyxiation of normal and I.A.A. poisoned nerve. Action current response to a short tetanus.

(2) *The greater susceptibility of* I.A.A. *nerve to oxygen want.*

Fig. 3 is one of four experiments on the rate of asphyxiation of normal and I.A.A. poisoned nerves which gave similar results. In such experiments frequent stimulation must be avoided because of the injurious effect of stimulation itself on I.A.A. nerve. In Fig. 3 the points plotted include all the test stimuli given, each being about 1 sec. tetanus by the induction coil. The poisoned nerve was completely asphyxiated in 1½ hours, while the normal nerve still gave considerable response after 2½ hours. The ratio of the times required to asphyxiate normal and I.A.A. nerves has not been determined exactly: approximating, however, it may be estimated that nerve poisoned with the concentration of I.A.A. used is asphyxiated in about one-third the time required for normal nerve.

One point to be particularly noted in Fig. 3 is the absence of recovery of I.A.A. nerve on readmission of oxygen. In the particular experiment shown oxygen was admitted 1 hour after complete asphyxiation of I.A.A. nerve. If this had occurred immediately after its failure, as in some other experiments, a certain amount of recovery would have occurred (see Table II). Apparently some irreversible process goes on which, past a point, makes oxidative recovery impossible.

The asphyxiation of nerve is primarily due to the depletion of the oxidation reserve, possibly in part also to the accumulation of toxic metabolites if such there be. It may be that I.A.A. nerve uses its oxidation reserve less economically, or that the products of disturbed metabolism are more toxic. It seems more likely that the formation of lactic acid with its attendant energy liberation, since anaerobic production of lactic acid by nerve is an established fact, helps to keep the nerve in working condition.

(3) *The effect of sodium lactate on nerve poisoned by* I.A.A.

For testing this point a phosphate-buffered I.A.A. solution of any required concentration is made up and divided into two portions, to one of which sodium lactate is added, usually in an amount to make the solution finally contain 180 mg./100 c.c. of lactate. Two symmetrical nerves are then soaked respectively in the two solutions. After 2 hours or more soaking, both are mounted in the chamber and continuous stimulation of both carried out simultaneously. Eight experiments of this kind were made, which are summarized in Table I, the nerve which had been soaked in I.A.A. solution containing lactate being always found to stand a considerably longer period of stimulation than its symmetrical

TABLE I.

No. of exp.	I.A.A. poisoning: Concentration p.c.	I.A.A. poisoning: Duration of soaking hr. min.	Time to complete exhaustion. Nerve previously in I.A.A. alone hr. min.	Time to complete exhaustion. Nerve previously in I.A.A. + lactic acid hr. min.	Concentration of lactate added mg./100 c.c.	Remarks
1	0·4	3 15	1 10	2 30	90	Stimulation by induction coil at about 50 makes and 50 breaks per sec.
2	0·4	3 20	1 15	2 15	180	
3	0·37	3 0	2 30	3 30	150	
4	0·37	3 50	1 20	2+	150	
5	0·37	3 40	1 10	1 40+	150	
6	0·4	3 5	2 20	3 20+	180	The lactate nerve in 180 mg./100 c.c. lactate for 3 hrs. before being poisoned.
7	0·4	2 5	3 0	4 30+	180	Slow stimulation at about 7 per sec.
8	0·4	4 15	2 10	4 30+	180	

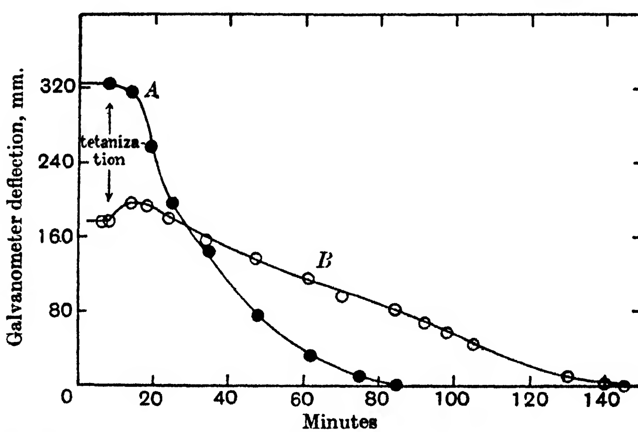

Fig. 4. The beneficial effect of sodium lactate on nerve poisoned by I.A.A. and continuously tetanized: *A*, by I.A.A. solution alone; *B*, by I.A.A. solution containing lactate in concentration 180 mg./100 c.c. Both in O_2. Response to a test stimulus during a short gap in the tetanus. Note that *B* endures tetanization much longer.

nerve poisoned in I.A.A. solution only. Fig. 4 shows one of these experiments, in which continuous stimulation was given by induction coil. The nerve soaked in I.A.A. alone failed completely after 1 hour 15 min., while it took 2 hours 15 min. to induce complete failure in the nerve treated with lactate as well as I.A.A.

There is no doubt about the beneficial effect of lactate on I.A.A. poisoned nerves in oxygen or air. In nitrogen, however, the effect is not obviously shown. Table II includes all the experiments made on this point. Of the eight experiments only two show a small difference in the time to complete asphyxiation in favour of the lactate nerve. On

TABLE II.

No. of exp.	Duration of previous soaking in I.A.A. solution hr. min.	Time to complete asphyxiation in min. Nerve previously in: I.A.A. alone	I.A.A. + lactic acid	Recovery on readmission of oxygen: p.c. of initial deflection: I.A.A. alone	I.A.A. + lactic acid	Remarks
1	2 25	60	60	0	36	Nerve continuously stimulated in N_2
2	2 45	60	60	29	60	
3*	2 50	45	45	0	0	
4	4 10	100	110	0	12	Nerve resting in N_2
5	2 35	110	110	33	71	
6	2 50	160	160	29	91	
7	2 45	135	150	22	60	
8	6 15	160	160	9	19	

N.B. The concentration of I.A.A. used in this series of experiments was always 0·4 p.c. and that of lactate 180 mg./100 c.c.

* In this experiment oxygen was readmitted 40 min. after complete asphyxiation.

readmitting oxygen, however, the beneficial effect of lactate again manifested itself. The nerve containing lactate recovered more rapidly and more completely (Table II).

Thus experiments both in oxygen and nitrogen demonstrate that nerves with the ability to form lactic acid, or even containing added lactate, have a greater working capacity. One may conclude that nerve is capable of utilizing lactic acid by oxidation and that the formation of lactic acid is normally of functional importance.

A few miscellaneous observations were made. The poisonous effect of I.A.A. in nerve, as in muscle, is irreversible. A nerve first soaked in 0·4 p.c. I.A.A. solution for 2 hours and then transferred to normal Ringer for 3 hours behaves as if it had remained in I.A.A. solution all the time. Indeed the effect is progressive. Even without stimulation a poisoned nerve will become inexcitable after 10 hours or so. In this connection a curious phenomenon was found. A pair of symmetrical nerves was soaked in the same I.A.A. solution for 2 hours, one nerve was mounted in air, the other was allowed to remain in the poison solution; it is found that the one in air becomes inexcitable considerably earlier than that kept in the solution. This statement is based on two experiments. Possibly there are toxic metabolities which accumulate more in air than in solution.

DISCUSSION.

The present work shows on the one hand that the formation of lactic acid is not essential to nerve function, yet on the other that it is required if the nerve is to endure prolonged activity. This is the same as in muscle.

There, too, lactic acid formation, though not essential to contraction, is responsible for the normal capacity of a muscle to do a great amount of work, and as recently shown by Mawson [1932], an I.A.A. muscle receiving lactate can give a much greater number of contractions than one without. Such findings suggest that lactic acid in nerve, as in muscle, may be an intermediate state in the oxidation of carbohydrates.

The present work, showing the functional importance of lactic acid in nerve, and the chemical investigations, which have given no evidence for such importance, are not necessarily in conflict. If lactic acid is oxidized as soon as it is formed during nerve activity, there need not be any accumulation of lactic acid as a result of stimulation; the accumulation, if any, represents the difference between formation and removal, which would most probably be too small to be detected. Carbohydrate content, however, should be decreased by stimulation in any case, a point which chemical study has so far failed to reveal. The following calculation, however, is pertinent. When a nerve is stimulated continuously at any frequency higher than about 100 per sec., the heat rate reaches its maximum within half an hour and then progressively decreases. With a lower frequency of about 50 per sec., and employing stimuli with optimal characteristics, namely, properly timed condenser discharges, a steady state of heat production is usually established, at the rate of about 30×10^{-6} cal. per g. per sec. [Beresina and Feng, unpublished data]. Let us assume that during activity nerve burns glucose to CO_2 and H_2O, and let us take the heat of combustion of glucose to be 3·7 cal. per mg. Assuming all the energy liberated by stimulation to come ultimately from carbohydrate, the production of heat at the rate of 30×10^{-6} cal. per sec. for a period of 3 hours would require the consumption of only 0·09 mg. of glucose. This estimate is in all probability too high, and it would seem at least an open question whether the change in carbohydrate content due to stimulation falls within the limits of experimental error of present-day chemical methods.

Hill [1932] had very recently shown that the course of recovery heat production in nerve can be closely represented by a double exponential equation with a quick and small first term and a slow and large second term. Possibly the first term represented anaerobic recovery by lactic acid formation, while the second term was oxidative recovery. If such were the case, the heat production of I.A.A. poisoned nerve should follow a different course from normal. This had been put to experimental test. Fig. 5 shows the analysis of the heat production in an 8 sec. stimulus by I.A.A. poisoned nerves. Just as the action current of such nerves pro-

gressively becomes smaller after increasing amount of stimulation, their heat production also decreases very rapidly in the course of an experiment. In the experiment shown, which is the better of two made, twenty 8 sec. stimuli were given at regular intervals of about 5 min. The response to the last stimulus was only about half as large as that to the first. Eight stimuli were about of the same size, and the analysis given is of the average of these only. Neither in absolute value nor in time course is the heat production of I.A.A. nerve significantly different from normal [see Hill, 1932].

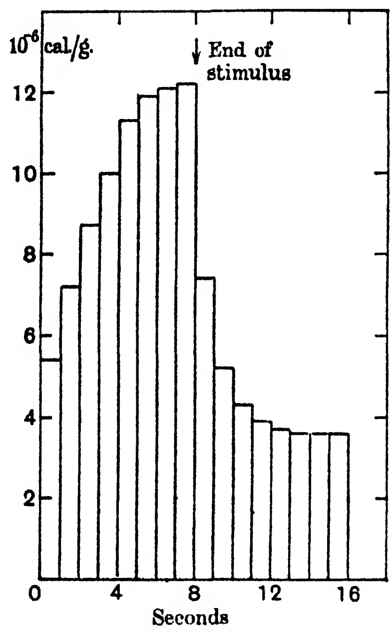

Fig. 5. Analysis of the heat production in an 8 sec. stimulus by I.A.A. poisoned nerve in O_2. It appears to be normal in shape.

The only previous investigation of which I am aware on the effect of I.A.A. on nerve activity is that of Ronzoni [1931], who reported that I.A.A. poisoned nerve stimulated twice every second is asphyxiated very much earlier in nitrogen than normal nerve similarly treated. The author, however, did not give any experimental details, not even the concentration of I.A.A. used, and it is difficult to appreciate fully the meaning of her results.

Summary.

1. Frog's nerve is definitely poisoned by soaking in 0·37–0·4 p.c. iodoacetic acid for 2 hours or longer. The poisonous effect shows itself in the inability of such nerve to endure prolonged activity in oxygen.

2. Such nerve is also much more readily asphyxiated in nitrogen than normal.

3. Giving sodium lactate to poisoned nerve improves its capacity for prolonged activity in air or in oxygen; in nitrogen the time to complete asphyxiation, with or without continuous stimulation, does not seem to be lengthened by the presence of lactate, except occasionally and slightly. On readmission of oxygen the superiority of the poisoned nerve containing lactate again manifests itself in its quicker and greater recovery.

4. These results indicate that frog's nerve is capable of utilizing lactic acid by oxidation, and that the formation of lactic acid, though not essential to nerve conduction, enables the normal nerve to perform long hours of continuous function.

I wish to express my sincere thanks to Prof. A. V. Hill for his interest in this work and for his encouragement; to Mr J. L. Parkinson I am indebted for much advice and assistance.

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A COMPARISON OF THE PROPERTIES OF CERTAIN TISSUE EXTRACTS HAVING DEPRESSOR EFFECTS.

BY RALPH H. MAJOR, J. B. NANNINGA AND C. J. WEBER.

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THE number of recent papers on the depressor substances in the various tissues of the body reveals an increasing interest in the subject; Frey [1929], Lange [1930], v. Euler and Gaddum [1931], Zipf [1931], Drury and Szent-Györgi [1929], and Felix and Putzer-Reybegg [1932]. It seemed desirable to compare the extract with which we have been working [1929, 1930] with the substances obtained by these workers.

In our experiments we have used extracts of brain, liver, lung and pancreas, prepared in an identical manner, although more experiments were carried out with brain extract than with the other extracts. The experiments on blood-pressure were carried out under light ether anæsthesia.

METHODS OF PREPARATION OF THE EXTRACTS.

Ten kg. of brain, liver, lung or pancreas were mixed with 10 l. of acetone and the mixture stirred vigorously for a period of an hour. After filtration the filtrate was evaporated to dryness and taken up with 200 c.c. of distilled water. This preparation will be referred to in the future as the crude extract of brain, liver, etc.

Crude extracts were thus obtained from various portions of the brain, and Fig. 1 shows that extracts from the basal ganglia are more active than extracts from certain other parts of the brain.

The effect of the various crude extracts after treatment with "norit" is shown in Fig. 2. This treatment, shaking up 25 c.c. of crude extract with 2 g. of norit, removes all the activity of the lung extract, 90 p.c. of the activity from the liver extract, 50 p.c. from the pancreas extract and only a small amount from the brain extract. Since this treatment removes histamine this is strong evidence that the activity of the lung

extract and most of the activity of the liver extract is due to histamine or histamine-like substances, as was shown by Best, Dale, Dudley and Thorpe [1927], and that the activity of the brain extract is not due to histamine.

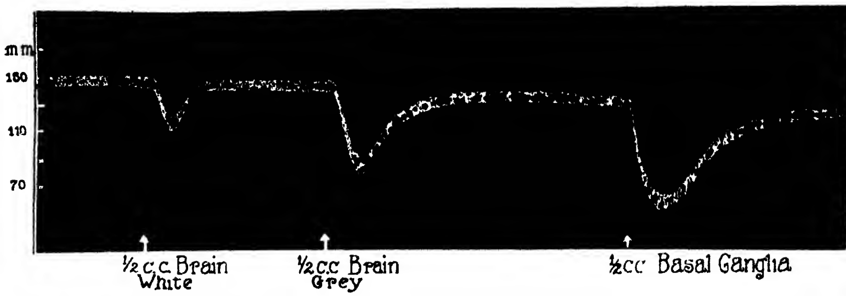

Fig. 1. Curves showing the depressor effect of extracts from various parts of the brain. 1 c.c. of extract represents 0·5 g. of brain tissue.

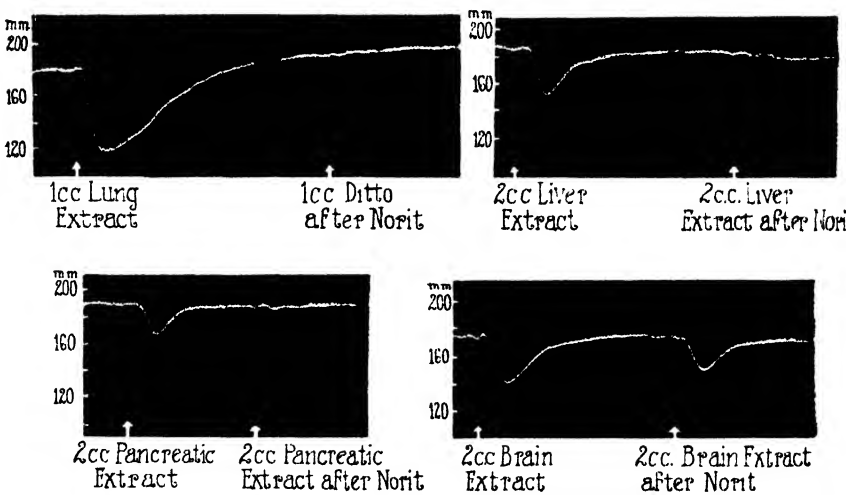

Fig. 2. The effect of treating the extracts with norit. Norit removes all of the depressor activity of the lung extract and most of the activity from extracts of liver and pancreas. Brain extract is only slightly affected by this treatment.

The behaviour of the crude extract after treatment with phosphotungstic acid is shown in Fig. 3. Treatment with phosphotungstic acid removes approximately 95 p.c. of the activity of the liver extract, 25 to 50 p.c. of that of the pancreatic extract, 95 p.c. of that of the lung extract and approximately 50 p.c. of that of the brain extract.

Phosphotungstic acid should remove histamine and choline and so evidence is furnished that the activity of brain extract is not due to these substances. Felix and Putzer-Reybegg, by fractional precipitation

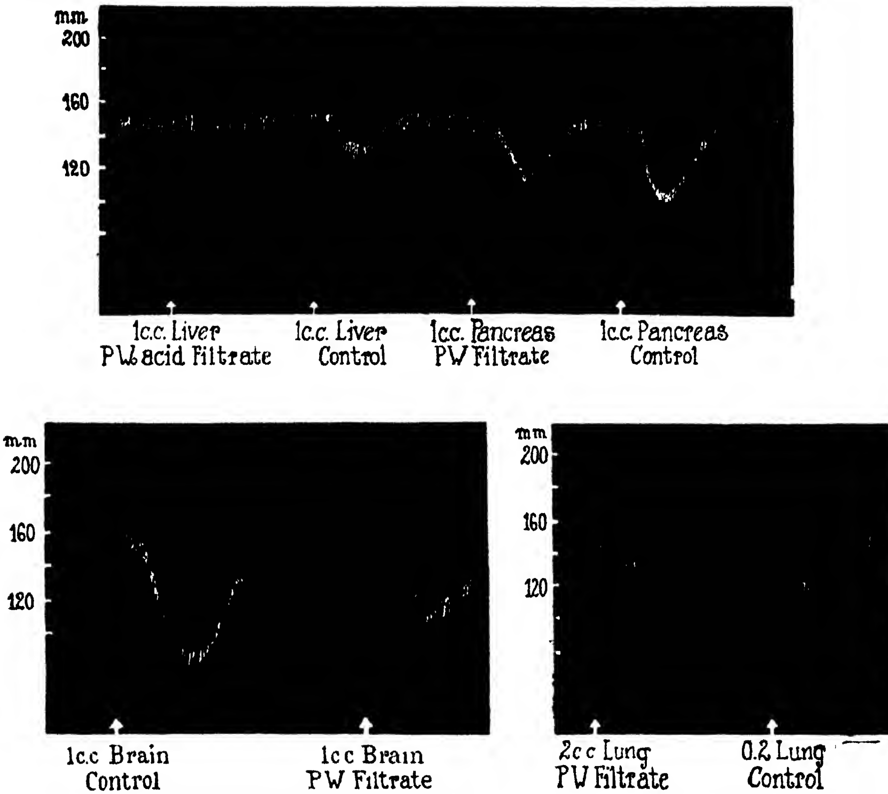

Fig. 3. The effect of treating the extracts with phosphotungstic acid. This treatment removes 95 p.c. of the depressor activity of the liver extract, 50 p.c. of the activity of the pancreatic extract, practically all of the activity of lung extract and less than 50 p.c. of the activity from the brain extract. Note that the amount of the lung extract control was only one-tenth of that used after treatment with phosphotungstic acid.

with silver, have brought evidence to show that 50 p.c. of the depressor activity of the precipitates from the kidney and mesentery was due to choline. Fig. 4 shows the effects of choline and of a silver filtrate of the brain extract, and that atropine abolishes the action of choline but has no effect upon that of the brain filtrate.

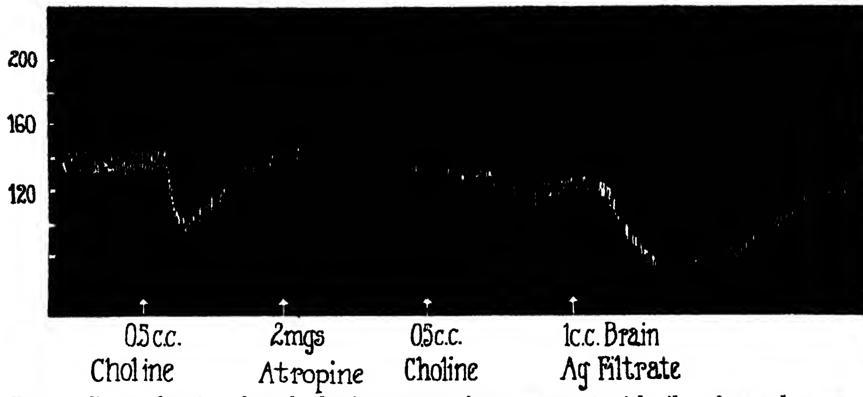

Fig. 4. Curve showing that the brain extract after treatment with silver has a depressor effect upon an atropinized animal.

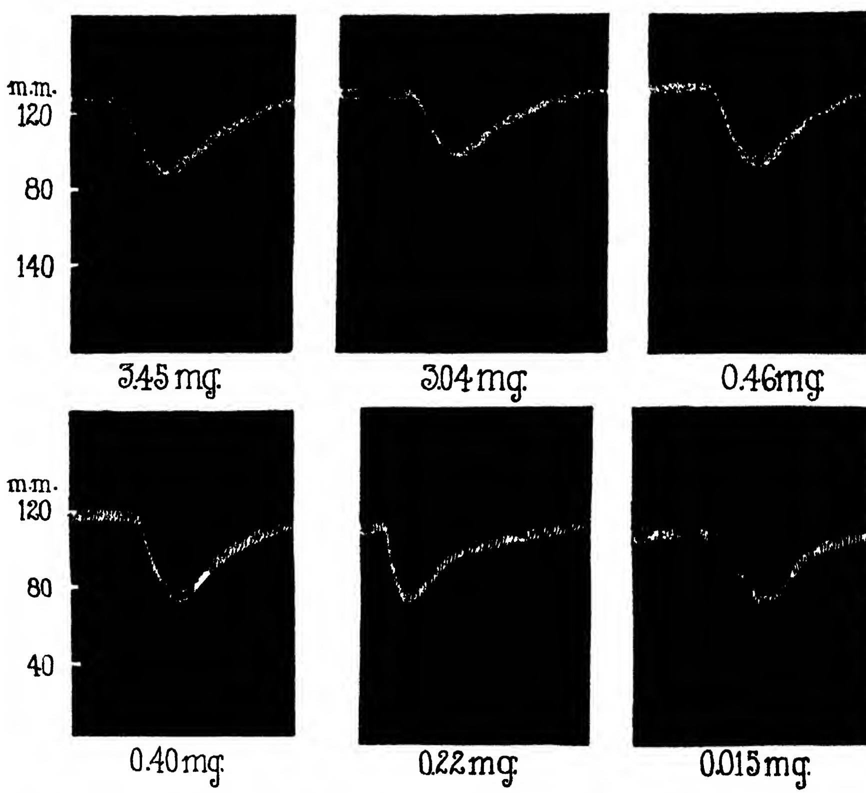

Fig. 5. Tracings showing purification of brain extract without loss of activity. Figures in terms of mg. of solids injected. The curve labelled 0·015 mg. shows the depressor effect of 0·015 mg. of histamine.

For the purification of the brain extract we have also proceeded as follows: the crude brain extract is treated with a saturated alcoholic solution of mercuric chloride and made alkaline; the abundant precipitate after removal of mercury can be divided into two fractions, one soluble in water, the other not; the active depressor principle with which we are working is in the former.

Fig. 5 shows the effects of purification upon the activity of the extract. The first curve shows the activity of the mercuric precipitate after removal of the mercury. The dose employed contained 3·40 mg. of solids. After treatment with phosphotungstic acid this extract shows the same activity when a dose containing 3·04 mg. of solids was injected. This solution was then made acid to Congo Red and treated with Lloyd's reagent, filtered, and the precipitate ground with barium hydroxide, water added, and again filtered. The barium was removed from the filtrate with sulphuric acid. The activity of an amount of the resulting solution which contained 0·46 mg. of solids is shown. When this solution was made alkaline and treated with norit, an amount of the filtrate containing 0·4 mg. of solids exhibits the depressor effect shown in the curve. This solution was evaporated to dryness and extracted with 90 p.c. alcohol, the alcoholic solution evaporated and taken up in water; an amount containing 0·22 mg. of solids has the activity shown in the curve, which is approximately equivalent to that produced by 0·015 mg. of histamine in aqueous solution.

We have also pharmacological evidence that these extracts do not owe their activity to either histamine or choline. In Fig. 6 the activity of the extract purified by treatment with Lloyd's reagent and alcohol is tested against a solution of histamine. The quantity of brain extract employed had about six times the depressor activity of the histamine used when tested on a dog under ether anæsthesia. The curve shows that the brain extract has no effect upon a virgin guinea-pig uterus, while the solution of histamine produced its characteristic response. This purified brain extract was next tested against the isolated intestinal loop of a rabbit. The intestinal loop showed the greatest response to choline, less to histamine, and showed no response whatever to the brain extract (Fig. 7).

Table I presents a comparison between the brain extract with which we have worked and the depressor substance described by other investigators. The "hormone" of Frey was isolated from the urine, the depressor substance of Felix and Lange was isolated from several organs, particularly the kidneys and the mesentery, while the depressor

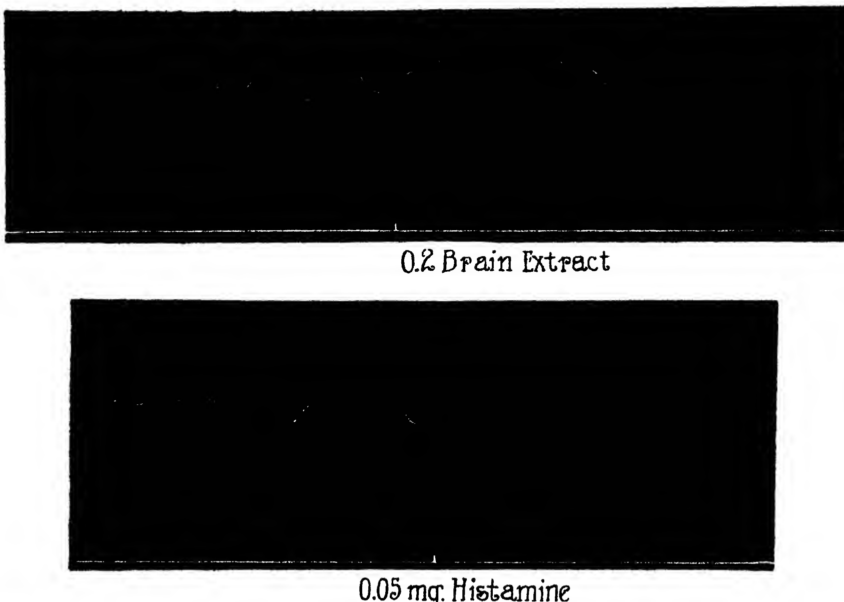

Fig. 6. Curve showing that the purified brain extract has no effect upon the virgin guinea-pig uterus, while histamine produces a characteristic contraction.

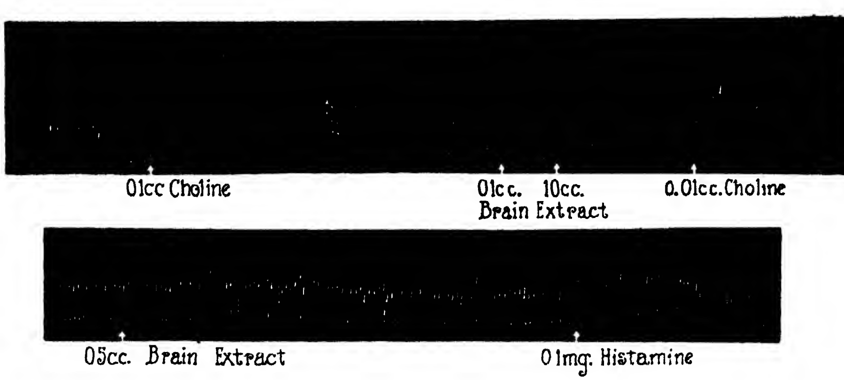

Fig. 7. Curve showing that the purified brain extract has no effect upon the isolated intestinal loop of the rabbit, while choline produces a marked contraction and histamine a lesser contraction.

extract of Euler and Gaddum was also obtained from several organs, extracts from the small intestine and brain apparently containing the largest amounts of the depressor principle.

TABLE I.

Treatment	Histamine	Choline	Adenosine	Frey's hormone	Euler and Gaddum	Felix and Lange	Brain
Silver precipitation	–	+	–	.	.	–	+
Phosphotungstic acid precipitation	–	–	–	–	–	–	+
Norit absorption in alkaline solution	–	.	.	.	.	.	+
Mercury precipitation in alcoholic solution (acid)	–	.	.	.	.	–	+
Mercury precipitation in alcoholic solution (alkaline)	–	–	–	.	–	–	–
H_2SO_4 5 p.c. Boiling 5 minutes	+	+	–	–	.	+	+
NaOH $N/1$. Boiling 5 minutes	+	.	+	–	–	.	+

+ or – indicates presence or absence of activity in the filtrate after indicated treatment

If the data given by these other observers are constant, we are apparently working with a different depressor substance.

SUMMARY.

The extract of brain tissue with which we have been working has a powerful effect. It is not precipitated by silver, phosphotungstic acid or mercury in acid alcoholic solution. It is not absorbed by norit from an aqueous solution, but is precipitated by mercury in alkaline alcoholic solution. It is not destroyed by boiling in 5 p.c. sulphuric acid or in normal sodium hydroxide for 5 minutes. It is active in atropinized animals and does not show a Pauly reaction. In one of our previous communications the statement was made that the purest solution we had obtained which contained this depressor principle showed a positive Sakaguchi reaction, indicating the presence of a guanidine compound. Since the publication of that paper we have obtained an extract which is quite active but gives a negative Sakaguchi reaction.

This work was aided by a grant from the National Research Council and the American Medical Association.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

July 2, 1932.

Isolation of components in the retinal action potential of the decerebrate dark-adapted cat. By RAGNAR GRANIT.

Leads from the cornea and decerebration wound have been taken to the input of a directly coupled amplifier with a string galvanometer in the bridge formed by two valves working in a "push pull" arrangement. The aim of the work has been to try to establish a biological solution of the well-known complex action potential of the retina. In view of the high degree of differentiation of the cells in the various layers of the retina, the plan adopted was to look for an agent capable of influencing some parts of the action potential without interfering with others. Ether was chosen first for various reasons and was found to be satisfactory.

Etherization of a preparation, stable for several hours, removes in three characteristic steps definite components of the response to stimulation with white light. These components are indicated in Fig. 1 by the Roman letters in the order of their disappearance. Process I (P I) disappears rapidly during progressive etherization and the fast components are left unchanged. It is essentially a high-intensity process. Thus at a certain early stage of anæsthesia the slow component may be minute or even absent at high intensities whereas the low-intensity action potential is almost or even completely unchanged. Therefore the slow so-called *c*-wave of the composite potential is not homogeneous (see Fig. 1). P II next becomes sluggish during continued anæsthesia and ultimately disappears. Finally only the negative P III is left, provided the intensity has been high enough to elicit a negative component. So far the changes are reversible. The last stage is a gradual disappearance of P III. The changes have then become irreversible.

Records from the optic nerve indicate that P II is the component chiefly responsible for the impulses. P III has not been found associated with the setting-up of impulses. P I *may* promote the discharge but appears to be much less effective than P II, if at all so. The optic nerve discharge does not cease on removal of P I. The nerve records were obtained with needle-electrodes [Adrian and Bronk] stuck into *foramen opticum*, the galvanometer serving as an integrator.

It has been possible to confirm the relative independence of the three components in experiments where for some reason or other certain components have been lacking from the beginning.

The analysis of the experimental evidence supports the conclusion that the three processes pictured in Fig. 1 are the normal components of the action potential of an animal possessing very few cones. The initial negativity (*a*-wave) and the off-effect (*d*-wave) are recognized to be small and inconstant in certain mammals including the cat. Their place in the solution of the composite effect can be seen in Fig. 1. The typical off-effect is a retardation in the drop of potential following the removal of the stimulus. It may then be visible in the record from the optic nerve.

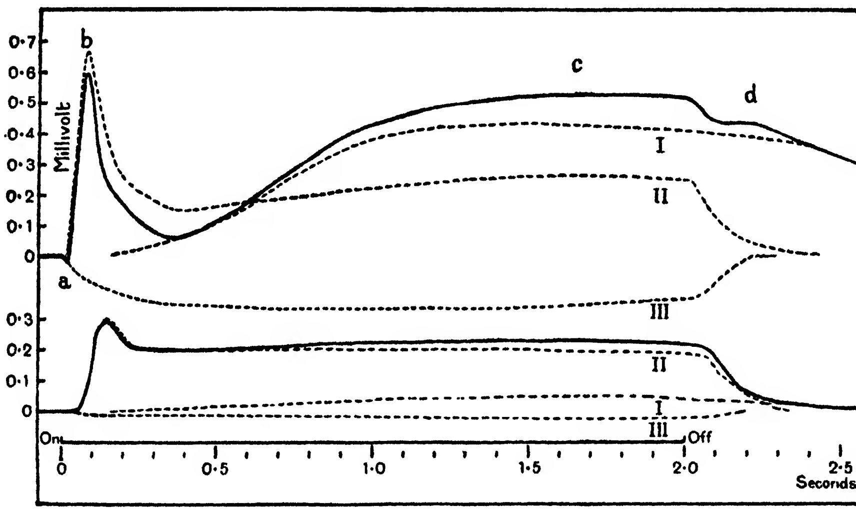

Fig. 1. Diagram showing solution of composite retinal action potential at two intensities of illumination in the ratio of 100 : 1. Broken lines: components. Lines drawn in full: action potentials as recorded; upper line in response to stimulus 100, lower line in response to stimulus 1. The *a*-wave is broadened slightly out of scale in order to show its derivation more clearly.

Observations on absence of cyanosis at great altitudes.

By Raymond Greene[1].

During the summer of 1931 I was a member of the expedition which made the ascent of Kamet, a mountain in the Himalayas 25,447 ft. in height. During the ascent I kept careful observation on the colour of the

[1] Schorstein Research Fellow, Oxford University. Medical Officer, British Himalayan Expedition, 1931.

lips and faces of my companions. We were all young, in good physical training, and well acclimatized to altitude.

Up to and including Camp V, which was pitched at a height of 23,300 ft., I observed no cyanosis, whether at rest or during exercise. Between this point and the summit I was, for the most part, climbing alone and therefore unable to observe. On the summit we encountered a strong and icy wind and we were all blue. But I do not think that we were bluer than we would have been under similar wind conditions at ordinary levels.

Barcroft and his colleagues found that natives of Cerro de Pasco (14,200 ft.) and Europeans who had lived there for a considerable time were distinctly blue. They report that members of the expedition were still blue after several days at this altitude, though less so than on arrival. On the other hand, after acclimatization members of the Pike's Peak Expedition were not blue, nor were those persons, such as the manager and the policeman, who had lived for three months on the summit. Members of the Pike's Peak Expedition did not become blue after short bouts of exercise. During long climbs cyanosis became very distinct, but it disappeared very rapidly on pausing.

Even during long climbs, however, I could detect no cyanosis in members of the Kamet Expedition. This was probably due to the fact that they were in far better training than members of the Pike's Peak Expedition, who were almost continuously occupied with laboratory experiments.

Release of extensor rigidity of the fore-limb by separation from lumbo-sacral segments. By T. C. Ruch. (*Oxford.*)

A post-brachial transection of the spinal cord which removes from the sphere of activity the lumbo-sacral segments augments many-fold the decerebrate rigidity in fore-limb extensors. The exaltation of rigidity is not due to irritation of ascending tracts by the cord lesion, for it occurs when the spinal cord has been severed weeks before examination. The phenomenon is considered an adorally acting "release of function" due to freeing the fore-limb reflex arcs from tonic inhibition originating below the level of section. *A priori*, such tonic inhibition would be expected to originate from the proprioceptive nerves from hind-limb muscles. But release of fore-limb extensor reflexes occurs when the portion of the cord removed has been previously deprived of a large share of its afferent paths by the nerve sections practised in preparation for reflex experiments on the hind-limbs. To follow out the implications

of this observation, the lumbo-sacral segments have been deafferented on both sides, decerebration performed, and, after recovery from the anæsthetic, the condition of fore-limb rigidity examined, either clinically in the intact limb, or by recourse to the optical isometric myograph and isolated triceps muscle. This more extensive reduction of the afferent paths which might act upon the reflex centres for the fore-limbs has been found in a series of experiments not to produce any detectable release. The stretch reflex of triceps muscle seems entirely normal in magnitude. When, however, transection is performed at the level of the highest spinal root severed, which eliminates only segments already deafferented, the usual exaltation of fore-limb rigidity occurs. Complete deafferentation is therefore not equivalent to cord section in freeing the fore-limb from inhibition. The ubiquitous possibility of excitation from the cut ends of spinal roots being the source of inhibitory impulses intrudes, although the data of Adrian [1930] suggest that the 20 hours allowed after deafferentation is ample time to insure complete freedom of the lumbo-sacral segments from afferent impulses. The conclusion reached is that a portion of spinal cord entirely deprived of centripetal impulses is still capable of restraining the activity of fore-limb proprioceptive reflex arcs. There appears to exist an adorally directed stream of inhibitory impulses of purely intracentral origin and such inhibition is, therefore, an automatic function of the spinal cord such as the automatic rhythmical functions under anæsthesia established by Graham Brown [1914].

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The dynamics of the ventricular contraction of the tortoise heart. By G. Stella. (*Univ. Coll. London.*) (*Preliminary communication.*)

The duration of the contraction of the ventricle is affected by a variety of conditions, among which the arterial resistance has long been recognized to play a considerable part. Thus O. Frank [1895] on the frog's heart, and De Heer [1912] and Patterson, Piper and Starling [1914] on the mammalian heart have observed that the higher the arterial resistance the longer the heart remains in a state of contraction. The mechanism, however, by which this affects the systole is not clear.

Experiments performed on the isolated ventricle of the tortoise heart

indicate that there is a tendency for the contractile stress set up in the heart muscle at any moment to be determined by its volume at that moment and by the time that has elapsed since the commencement of systole.

These were made at a low temperature (8°–10° C.) on ventricles driven artificially 6 to 10 beats per minute. The ventricle was perfused with a mixture of Ringer's solution and tortoise blood; changes in volume and in intra-ventricular pressure were recorded, the latter optically. The apparatus employed was similar to that used by Peserico [1928] modified so as to allow of changing the contraction from isotonic to isometric, and *vice versa*, at will. Under these conditions, and while the heart was working regularly and isotonically, input and output were cut off at the end of a systolic ejection so that the next systole should occur isometrically at the smallest volume reached by the preceding isotonic systole. The last part of the isometric contraction pressure curve was then found to coincide very well, within the limits of experimental error, with the last isometric part of the preceding isotonic systole; both curves, moreover, reached the zero line at about the same time from the beginning of the systole. Similar results were obtained with all arterial resistances investigated, and with very different volumes. In some experiments the systolic ejection was interrupted at different times from the beginning, so that the remaining part of the systole should occur isometrically, and the heart made to contract at the next systole isometrically at the volume it had at the time when the interruption was effected. Here, also, the second part of the two curves for intra-ventricular pressure showed a very satisfactory coincidence in course and time. All these facts are made clearer by the fact that variations in duration of systole for different volumes may be very considerable. The conclusion seems to be that the volume of the heart is a determining factor for the energy liberation not only at the moment of stimulation but throughout the whole systole. Quick release experiments were also performed by withdrawing a known amount of liquid from inside the heart at different moments from the beginning of an isometric systole. The fundamental findings described by Gasser and Hill [1924] on the redevelopment of tension for the striated muscle of the frog were found to repeat themselves here; according to Gasser and Hill, however, if the release takes place after the peak of the contraction, redevelopment does not occur. Here, however, redevelopment was found to take place even if the release was made as late as the second third of the downward part of the curve. Whenever the release was made not too abruptly the last part of the

contraction was found, moreover, to follow the course corresponding to the new volume.

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The effect of insulin on the relation between the oxidation and synthesis of sugar. By E. W. H. Cruickshank. (*Dept. of Physiology, Dalhousie University, Halifax, N.S.*) (*Preliminary communication.*)

The question as to whether or not insulin increases oxidative metabolism has been much debated. The discrepancy between results recorded has arisen from the difficulty in recording accurately the gaseous metabolism of a skeletal or cardiac muscle preparation and of estimating the changes in the glycogen content of the tissue under observation.

By an improved method for the estimation of oxygen and CO_2 it has been found that as far as the isolated heart is concerned hyperglycæmia produces a 10 p.c. increase in oxygen consumption with an equivalent increase in heat production, the respiratory quotient being unity. The addition of insulin (10 to 20 units of Iletin; Eli Lilly & Co.) in hyperglycæmia increases the oxidative changes by only 3 p.c., it alters the balance between oxidation and synthesis from a percentage normal ratio of 98 : 2 to one of 70 : 30, with the result that glycogen synthesis is increased some 30 p.c. Such a change is associated with a marked increase in the removal of sugar from the circulating blood. Similar experiments have been carried out with hearts subjected to a rapidly progressive hypoglycæmia, and the general trend of results is such that one is forced to the conclusion that, even with a rapid depletion of blood sugar, insulin still exerts a powerful synthetic action. In some hearts there is a distinct storing of glycogen, in others a definite sparing of carbohydrate stores. Oxidative processes may show the usual 3 p.c. increase or may suffer diminution. In conditions of hypoglycæmia where all the blood sugar used and a certain amount of muscle glycogen are oxidized, insulin changes the usual oxidation synthesis ratio from 100 : 0 to an average of 85 : 15.

Adrenaline and reflex excitability of the cardio-inhibitory centres. By G. STELLA. (*Univ. Coll. London.*) (*Preliminary communication.*)

The question whether adrenaline has any effect upon the cardio-inhibitory centres remains still an open one since Oliver and Schäfer's [1894] first observations of a marked slowing of the heart rate following its intravenous injection.

Among recent workers, C. Heymans [1930] explains the bradycardia as a direct consequence of the primary rise of arterial pressure in the sensitive regions of the arch of aorta and carotid sinus; he points out, in fact, that if the depressor and carotid sinus nerves be cut, slowing of the heart rate from adrenaline is no longer observed (that is, if the dose of adrenaline be not too large). On the basis of such results Heymans emphatically denies that adrenaline in the usual doses has any effect upon the cardiac vagus centres.

On the other hand, experiments by independent perfusion of the head in the whole dog [Anrep and Starling, 1925], or in the innervated heart-lung preparation [Anrep and Segall, 1926; Volhard, 1930] have shown that adrenaline injected into the separate head circuit, and thus incapable of affecting directly the systemic arterial pressure, usually still causes slowing of the heart, both when the pressure in the carotid sinus is maintained artificially constant or after complete denervation of the latter, as in Volhard's experiments. The difference in the results of Heymans and those of Volhard is surmised by the former to be due to the abnormal conditions of the head perfused with defibrinated blood in the latter's experiments. It seemed, however, that a more fundamental difference between the two kinds of experiment lies in the fact that Heymans cut the afferent paths through which the arterial pressure regulates the heart-rate thus making it impossible to detect an action (if any) of adrenaline upon the responsiveness of the cardio-inhibitory centres to the afferent impulses from the aorta and carotid sinus.

The conclusion reached from the present investigation is that adrenaline actually does affect the reflex excitability of the cardio-inhibitory centres, in the sense that the degree of slowing of the heart produced reflexly by increasing the pressure in the carotid sinus or the arch of the aorta is far more pronounced when adrenaline is circulating in the head.

The experiments were performed on dogs anæsthetized by morphine (0·5 cg.) and urethane (0·3 g. per kg.); to study the carotid sinus reflexes, this was isolated and perfused on both sides with a technique

already described, and the depressor nerves were cut, leaving the vagi intact. It was found that after the intravenous injection of adrenaline (0·5–1 c.c. of 1:30,000 solution in a 10 kg. dog) a rise of the perfusion pressure in the carotid sinus was much more effective upon the heart rate than before the injection, and the effect coincided in time with the bradycardia observed in the intact animal. The same results were obtained by altering the pressure in the aorta in the innervated heart-lung preparation: in these experiments the head was perfused artificially by means of a Dixon pump, from the brachiocephalic, after careful denervation of both carotid sinuses. The slowing of the heart produced by increasing the systemic pressure was considerably more marked after injection of adrenaline in the circuit of the head. This was true also when, to counteract the effect of adrenaline vaso-constriction, the input from the pump was diminished so as to keep the pressure in the carotid arteries constant.

The marked bradycardia following an intravenous injection of adrenaline seems therefore to have as its cause the action of increased arterial pressure upon a more excitable cardio-inhibitory centre.

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Crystalline preparations with vitamin B_4 activity. By H. Barnes, J. R. O'Brien and V. B. Reader. (*Dept. of Biochemistry, Oxford.*) (*Preliminary communication.*)

A yield of 20–30 mg. of crystalline hydrochloride per cwt. of bakers' yeast has been obtained. The daily requirement of the adult rat is 0·01 mg. per day. (For method of assay see Reader [1930].) The preliminary extraction of the yeast and adsorption of the vitamin B_4 upon charcoal (pH 1) has been described elsewhere by Peters [1931]. This charcoal was extracted with 50 p.c. acid-alcohol, the alcohol removed *in vacuo*, and the extract subjected to successive treatments with mercuric sulphate, baryta, H_2S, and sodium phosphotungstate. A definite crystalline phosphotungstate, precipitated at pH 2, was obtained and recrystallized from 50 p.c. alcohol. It was then dissolved in 50 p.c. acetone to remove the phosphotungstic acid with baryta. The filtrate,

after removal of acetone, was hydrolysed by heating on the water bath for 1 hour with 5 p.c. HCl; then concentrated *in vacuo* to quite small volume (5 c.c. per cwt. original yeast). On addition of alcohol and ether and standing overnight crystals appeared. Fine needles, with M.P. 248° C. with charring. Preliminary analyses suggest the empirical formula of the anhydrous substance to be $C_4N_4H_5Cl$.

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The action of the vagus on the rhythm of the mammalian heart.

By G. L. BROWN, J. C. ECCLES AND H. E. HOFF. (*Physiological Laboratory, Oxford.*)

Experiments have been performed with a view to investigating the effect of vagus stimulation on the rhythmic discharge of the nodal tissue of the mammalian heart.

The action potential of the sino-auricular node of the decerebrate cat was recorded with an amplifier and Matthews' oscillograph, as previously described by Eccles and Hoff [1931]. Single break induction shocks were applied to the peripheral ends of the right and left vagus through shielded electrodes in the neck.

A single volley so set up in either vagus effects a distinct slowing of the rhythm lasting not more than ½ min. The right vagus is usually more potent than the left. The effectiveness of the vagal volley upon that nodal cycle during which it falls depends upon its position in the cycle. For instance, the nodal cycle is not lengthened when the stimulus to the vagus is applied at a short interval before the next nodal discharge, though subsequent cycles are lengthened normally. This ineffective interval which follows the setting up of a vagal volley we have, for the following reasons, regarded as representing the true latent period of the vagal inhibition. Its value remains constant in any one animal, provided the temperature of the animal is constant. Its length appears to be independent of the duration of the nodal cycle, since it is not significantly changed by alterations in the length of the nodal cycle produced by the following means:

(*a*) Concurrent tetanic stimulation of the other vagus.

(*b*) Concurrent tetanic stimulation of the accelerantes.

(*c*) When the vagal stimulus is timed to fall during the lengthened (compensatory) cycle following an induced extra-systole.

The value of the latent period varies between 100σ and 160σ in various experiments and is the same for maximal and submaximal stimulation of the vagus.

The time course of the inhibition (Fig. 1, lower curve) shows a sharp maximum which follows so closely on the end of the latent period that it involves the first inhibited cycle. A second very flattened maximum may occur several cycles later.

The inhibition produced by a second volley, either in the same or opposite vagus, sums with that produced by the first. In Fig. 1, for example, the upper curve shows the time course of the inhibition produced by a second volley set up in the same vagus 390σ after the first. There is summation of the inhibitory effects produced by simultaneous stimulation of the opposite vagi.

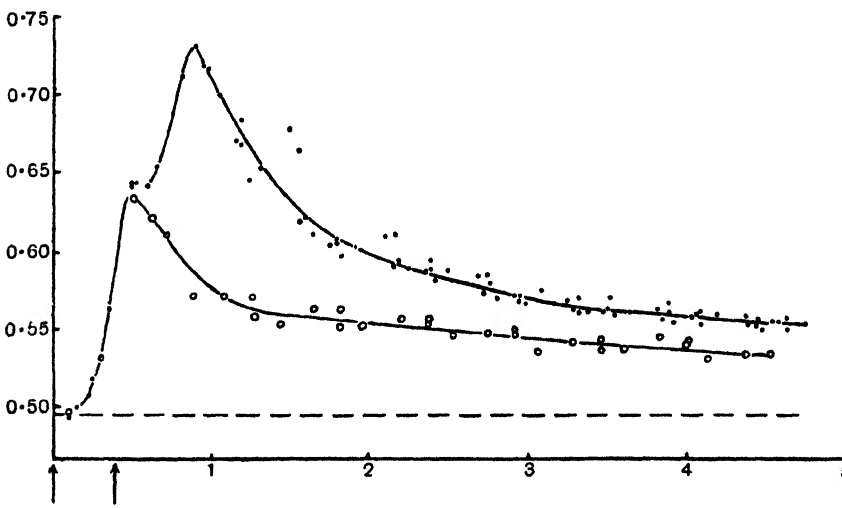

Fig. 1. Time course of inhibition following single (lower curve) and double (upper curve) stimuli applied to the right vagus. Times of the stimuli are shown by the arrows. Ordinates—duration in seconds of each nodal cycle. Abscissæ—interval in seconds between stimulus and end of corresponding cycle.

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Inhibition in peripheral preparations of skeletal muscle.
By GRACE BRISCOE.

In reflex preparations inhibition is demonstrated against a background of existing contraction, and shows itself as a loss or reduction of contractile tension. In peripheral preparations a sudden change in the rate of the stimulating current produces, under certain circumstances, a loss or reduction of existing tension, and this fall of tension may be more rapid than that due to cessation of stimulus.

The double reaction of reciprocal contraction and inhibition in antagonistic muscles can be shown if certain experimental conditions as to anæsthesia, differences in rate and strength of the stimulating currents, be observed.

If a muscle be maintained for a short period (*e.g.* 10–20 seconds) in a state of strong postural contraction by a suitable slow rate of stimulus applied to the peripheral nerve trunk, then a sudden change to a fast rate (*i.e.* one capable of carrying out phasic movements) will produce a sudden relaxation, although this same fast rate is of such a character that it would have produced a full contraction in the resting muscle. On the other hand, if a muscle be maintained in a condition of mild postural contraction, a sudden increase of rate of stimulation will augment the existing contraction. In other words, the result of a given stimulus is dependent on the receptive condition of the muscle concerned; if passive —contraction; if engaged in a different kind of functional activity—relaxation.

An extensor muscle is prepared in one limb and a flexor on the other side, so as to avoid interference. Neon lamp discharges are used as the source of stimulation [Briscoe and Leyshon, 1929]. The two nerves always receive the same rate of stimulation, whatever that rate may be. The strength of the stimulating currents is regulated by separate potential dividers, and is varied to either nerve according to the conditions of the experiment.

The neon lamp is made to flash at a slow *P* (postural) rate [Briscoe, 1931]. The strength of potential applied is so arranged that extensor develops a strong postural contraction, flexor a weak postural contraction. A sudden increase to an *M* (movement) rate now causes opposite effects in the antagonistic muscles. Extensor relaxes while flexor augments. Changing back to a *P* rate causes extensor to contract, while flexor relaxes. (See Fig. 1.)

If conditions as to strength of stimulus be reversed, so that flexor is placed in strong postural contraction, change to an *M* rate produces relaxation in the flexor and augmentation of contraction in the extensor. Similar muscles can be prepared, *e.g.* quadriceps in either limb, and made to show reciprocal contraction and inhibition.

It is improbable that these reactions take place in the nerve trunk at the site of stimulation, for they are affected by drugs introduced into the circulation. It is also improbable that they are confined to the neuromuscular junctions, because the same type of reaction can be demonstrated in muscles which are stimulated directly. The changes, however, are not so extensive as with indirect stimulation, probably because it is difficult to stimulate the whole muscle directly.

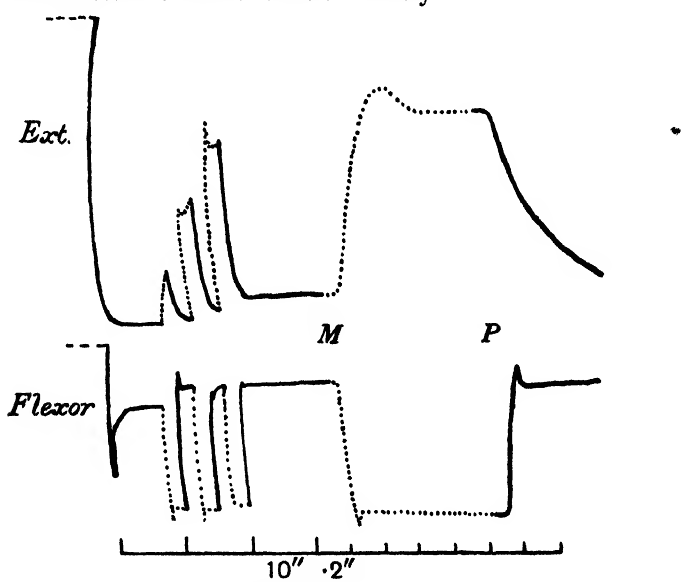

Fig. 1. Cat. Dial and ether. Tracing from record. Upper line right quadriceps, lower left tibialis and ext. l. dig. Contraction downwards. Whole line indicates when rate suitable for posture used (*P*), dotted line when rate suitable for movement used (*M*). Rates changed by plugging ½ megohm resistance in and out of charging circuit. No change in strength or character of stimuli. Extensor receives maximal, flexor submaximal *P* stimulus. First three spells of *M* stimuli recorded on slow drum, fourth on fast drum. Inhibitory effect not so marked in early stages of a posture.

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Normal gas tensions in the mucous membrane of the rabbit's uterus. By J. ARGYLL CAMPBELL. (*National Institute for Medical Research.*)

The following experiments were carried out for Dr Arthur Walton of the School of Agriculture, Cambridge, who requested information on gas tensions within the uterus.

Six rabbits (2–3 kg.) were anæsthetized with amytal and in addition a little A.C.E. mixture was required for three of them during the operation. In this the abdomen was opened for about 3 inches just above the symphysis pubis and small clips were placed at the free ends of the Fallopian tubes to close their openings into the abdominal cavity. A ligature was placed either at the internal or external end of the vagina. Up to 20 c.c. of oxygen and nitrogen gas mixtures, with oxygen tensions varying between 0 and 50 mm. Hg, were injected into the main cavity of the uterus by means of a hypodermic needle and gas container. After closing the abdomen, the lower half of the animal's body was kept under saline at 38 to 40° C. Samples (2 to 10 c.c.) of gas were withdrawn from the uterus usually at hourly intervals up to about the fifth hour after injection, and the oxygen and carbon dioxide tensions were calculated in the usual way after analysis in the small or large Haldane analyser. The results are recorded in Fig. 1, at zero hour, the tensions of the injected gases being shown. Anæsthetics as used in these experiments decrease the oxygen tension and increase the carbon dioxide tension on an average by 4 mm. Hg, so that allowance must be made for this. The numbers in Fig. 1 refer to the different experiments. Where the lines become horizontal, equilibrium between the gas tensions in the free gas in the uterine cavity and those in the cells of the mucous membrane has become established. Straight lines and not curves have been drawn between the points to distinguish the different experiments. Of course the carbon dioxide tension rises much more steeply than shown in most of the experiments.

The oxygen tension in five of the rabbits is obviously somewhere between 30 and 45 mm. Hg. In the remaining case, after allowing for effect of anæsthesia, the oxygen tension is about 20 mm. Hg, so that for all the rabbits investigated the limits for oxygen tensions are 20–45 mm. Hg.

In all six experiments the limits for carbon dioxide tensions are 40 and 60 mm. Hg. The point above 60 is probably due to anæsthesia.

These results are in general agreement with the figures for gas tensions in many other tissues of the body [Campbell, 1931].

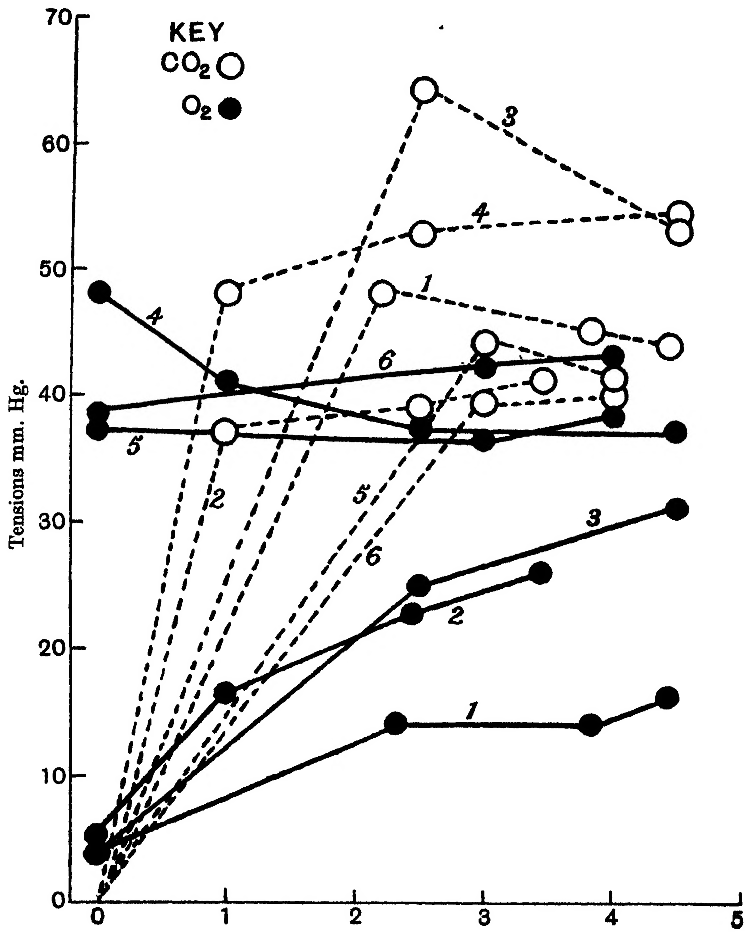

Fig. 1.

Similar results were obtained with the uterus of the cat, injecting only a bubble of gas, which was analysed in a Krogh micro-apparatus using 75–80 per cent. glycerol in water to enclose the bubble.

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Humoral transmission and the chorda tympani.

By O. S. Gibbs[1] and J. Szelöczey[2].

A considerable bulk of evidence has now accumulated to show that when a para-sympathetic nerve is stimulated a substance is liberated which possesses the pharmacological properties of acetyl choline, and which is probably the physiological connection between the nerve and its end organ. It is now certain that physostigmine acts by preventing the rapid normal destruction of this substance. This latter discovery makes it easy to demonstrate the production of the para-sympathetic hormone which we have been able to obtain in a relatively clean solution by a special method of perfusion from the submaxillary glands of both cat and dog. Essentially the experiment consists of perfusing the gland only for short periods during which the chorda tympani is stimulated, and immediately restoring the normal blood stream. After small quantities of physostigmine have been administered one may wash out from the gland during chorda stimulation a highly active substance. This substance we call "Chordastoff." Tested by injecting into the gland artery, "Chordastoff" shows itself to be a powerful secretory stimulant; on the blood-pressure it has a powerful depressor effect; to the isolated frog's heart it is depressant, and to the isolated rabbit's intestine, stimulant. In all four places this action is prevented by atropine, and both qualitatively and quantitatively it behaves like acetyl choline. Furthermore, the substance is rapidly destroyed by blood, or blood cells, which destruction is prevented by physostigmine. We are therefore able to confirm on the gland the work of Lœwi on the isolated frog's heart, and that of Engelhart on the eye. We also establish beyond any doubt the presence of this substance in the gland during stimulation of the chorda tympani.

These experiments were performed in the Pharmacological Institute, University of Vienna.

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The depressor constituents of muscle extracts.

By A. N. Drury[1].
(*Department of Pathology, Cambridge.*)

If a neutralized trichloracetic acid extract of ox heart muscle is introduced intravenously into the rat the heart rate is temporarily ... ed. This bradycardia appears to be an A.-V. rhythm, and sets in and ends abruptly. A similar disturbance is produced by injecting muscle

[1] Working on behalf of the Medical Research Council.

adenylic acid. By comparing the duration of the effect produced by the muscle extract with that produced by very small doses of muscle adenylic acid an estimate can be made of the amount of the responsible substance or substances. About 100 mg. of substance per 100 g. of fresh tissue are found to be present. On the other hand, the same muscle extract produces a heart block in the guinea-pig which has been shown to be due to adenylic acid [Drury and Szent-Györgyi, 1929; Bennet and Drury, 1931]. By comparing the effect in this animal with small doses of adenylic acid, the muscle extract contains only about 50 mg. of adenylic acid per 100 g. of fresh tissue.

The results suggest that a substance is present in addition to adenylic acid which has no influence upon the guinea-pig's heart and produces an A.-V. rhythm in the rat's heart.

The purification of the muscle extract on the lines already reported [Drury and Szent-Györgyi, 1929] shows that the barium salt of the additional substance is soluble in water while the lead salt is insoluble. As the same salts of adenylic acid have similar properties, it would appear that the additional substance is allied to adenylic acid. It is, however, less readily inactivated by hydrochloric acid than adenylic acid

Cytidylic acid, derived from yeast nucleic acid, in doses of 2 mg. and under, does not disturb the rhythm of the guinea-pig's heart and produces an effect in the rat identical with that seen after introducing muscle adenylic acid. A mixture of pure adenylic acid and yeast cytidylic acid has the same characteristics as the muscle extract, for if it is compared in the guinea-pig with known doses of adenylic acid it is less active than when it is similarly compared in the rat.

As cytidylic acid is more resistant to mineral acids than adenylic acid, it would seem reasonable that this, or some closely allied substance is the additional factor present.

Yeast cytidylic acid, introduced intravenously in doses of 1·0 mgm into the rabbit, causes a fall in blood-pressure of about 30 mm. Hg which is not abolished by atropine. From the above experiments it would appear probable that cytidylic acid is present in muscle extracts and plays a part in their depressor activity. It may be the depressor substance reported [Bennet and Drury, 1931] in the hydrolysat from the hydrolysis of yeast nucleic acid which was inactivated with difficulty by hydrochloric acid.

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